

Genetic structure and north-south decrease of genetic diversity in the Patagonian maqui berry (*Aristotelia chilensis* [Molina] Stuntz) determined by SSR marker analysis

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Research Article

Keywords: Conservation, demographic history, Elaeocarpaceae, management, microsatellite, phylogeography, quaternary glaciation

Posted Date: May 27th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1686258/v1>

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Conservation Genetics on May 27th, 2023. See the published version at <https://doi.org/10.1007/s10592-023-01526-1>.

Abstract

Maqui (*Aristotelia chilensis* [Molina] Stuntz) is a small endemic Patagonian tree that is currently being domesticated actively because of its edible berries, which have extremely high polyphenol content and anti-oxidant capability. Maqui can be easily propagated and is an efficient colonizer of deforested habitats to the point of often becoming an invasive species. However, little is known about its population structure and evolutionary history, limiting the design of conservation and domestication strategies. Genetic diversity of 14 populations (183 samples) from throughout the geographic distribution of the species in Chile was assessed with 13 polymorphic microsatellite markers. Samples were assigned to clusters based on Bayesian genetic and spatial structure analyses and the results were used to reconstruct patterns of phylogeographic and demographic history. We found that maqui populations are well-structured, with a dramatic reduction of genetic diversity from north to south, most notably in areas that were covered by ice during the quaternary glaciation. Three main genetic groups were revealed by Structure analysis. In conclusion, a clear pattern of genetic diversity reduction from its northern limit in central Chile to the Patagonian region was revealed, suggesting that an active recolonization process took place during the last few millennia following the last glacial period. These results will help to define the identification of accessions from different regions and contribute to support conservation and domestication initiatives.

Introduction

Patterns of genetic diversity of plant populations are influenced or modulated by intrinsic characteristics of the populations and by environmental conditions occurring at different temporal and spatial scales (Vucetich and Waite 2003; De Kort et al 2021). Contemporary climate models of the distribution and abundance of species (Huntley et al 1989; Osland et al 2017) now often include the distribution of genetic diversity within and among populations (Huang et al 2014; Ignazi et al 2019). They also often address the possible impacts of past climatic changes during the Quaternary that produced profound alterations in the distribution of plant populations and species, especially at high latitudes in both Hemispheres (Hewitt 2004; Fraser et al 2012). These impacts were especially important in southern South America during the last glacial period (LGM 26,000 YBP [years before present]; Moreno et al 2015) that resulted in a drastic reduction in temperature, high rainfall and large masses of ice forming on both sides of the southern Andes Range (Clapperton 1994). This glacial period generated the extinction of local populations and the contraction of ranges towards the north to more suitable refugia areas in mid and low elevations of the Coast Range and to favorable microclimates at higher latitudes including the western side of the Andes, as supported by palynological (Villagrán 1991), geomorphological (Veit and Garleff 1996) and genetic evidence (Premoli et al 2010; Marchelli and Gallo 2006). At the end of the last ice age, as the temperature increased and the great masses of ice melted, there was rapid recolonization from refuge areas (Villagrán 1991; Villagrán and Hinojosa 1997). Consequently, the current distribution and diversity patterns of plant species in the south-central zone of Chile are a direct consequence of past climatic oscillations that mainly occurred during the Quaternary and that are linked with glacial

displacement processes and mainly post-glacial recolonization at the beginning of the Holocene, 10,000 years BP (Abarzúa et al 2004; Villagrán and Hinojosa 2005; Moreno et al 2015; Maldonado et al 2022).

Phylogeographic studies of cold-tolerant tree species in the temperate forests of South America found areas with high genetic diversity in high latitudes, indicating that during the glacial period populations of these species survived in periglacial areas (Premoli et al 2010). Documented examples of this phenomenon include coniferous species such as *Fitzroya cupressoides* (Allnutt et al 1999; Premoli et al 2000), *Austrocedrus chilensis* (Pastorino et al 2004) *Pilgerodendron uviferum* (Premoli et al 2002; Allnutt et al 2003), *Araucaria XXXruticos* (Bekessy et al 2002) and *Podocarpus nubigena* (Quiroga and Premoli 2010), and angiosperms such as *Nothofagus alpina* (Marchelli et al 1998; Marchelli and Gallo 2006), *N. pumilio* (Mathiasen and Premoli 2010; Premoli et al 2010) and *Embothrium coccineum* (Souto and Premoli 2007; Vidal-Russell et al 2011). In contrast, a genetic diversity study in the mesothermic species *Eucryphia cordifolia* found high genetic diversity in the central coastal portion of its range (around 40°S), decreasing sharply northward and more moderately southward, suggesting that this area was the main refuge during glaciations (Segovia et al 2012). However, genetic diversity studies for cold-sensitive plant species of the South American temperate forest are practically absent.

Aristotelia chilensis (Molina) Stuntz (Elaeocarpaceae), locally known in Chile as maqui, is a dioecious evergreen shrub or small tree native to continental Chile and Argentina. Maqui has a wide distribution in Chile, from 32°S to 45°30'S and from sea level to 1,500 m altitude (Rodríguez et al 2018). There is no experimental evidence on the physiological responses of maqui trees to frost or cold conditions. Based on horticultural experience, Huxley (1992) proposed that maqui was frost-sensitive. However, Thomas (1992) suggested that maqui are only cold hardy in the milder and moister areas of the temperate zone, tolerating temperatures down to around -10°C. Nevertheless, genetic differentiation linked with a specific latitudinal cline has thus far not been described.

Maqui has high levels of phenotypic plasticity, with different growth habits ranging from shrubs in the north to trees towards the south (Lusk 2002). In Chile and Argentina, *A. chilensis* has been described as a representative understory species in sclerophyll shrublands and deciduous *Nothofagus* forests, often in conjunction with *Schinus patagonicus*, *Lomatia hirsuta* and *Berberis spp.* (Bravo et al 2015). Maqui also has an important ecological role, often acting as a colonizer of previously deforested lands and disrupted soils (i.e. pioneer species or early successional species), mitigating the effects of erosion while forming wide monospecific associations known as “macales” with microhabitats for other plant seedlings (Hoffmann 2005). Its main mode of seed dispersal is through endozoochory by birds such as *Elaenia albiceps*, *Patagioenas spp.*, *Turdus falcklandii*, *Pyrope pyrope* and *Curaeus curaeus*. *E. albiceps* may be especially important, as they are most abundant during the summer when the maqui fruit ripens (Bravo et al 2015; Donoso Zegers 2006). Maqui is a major concern in the Juan Fernandez Archipelago, because as an invasive species it has displaced endemic flora throughout the island (Smith-Ramirez et al 2013).

A. chilensis belongs to the family Elaeocarpaceae, which includes around 644 species distributed in at least nine recognized genera (The Plant List 2013). Current evidence, including diverse molecular data

(ITS and universal chloroplast data from several Elaeocarpaceae species), supports the hypothesis that *Aristotelia* species have a “southern plant pattern” model of distribution (Sanmartín and Ronquist 2004). The genus *Aristotelia* originated in Australia ca. 67 Ma (Paleocene) and later evolved and diverged into *A. chilensis* (southern South America, during the Oligocene ca. 24–27 Ma), *A. fruticosa* and *A. serrata* (New Zealand, during the Pleistocene ca. 3 Ma) through dispersion mechanisms. Since *Aristotelia* divergence occurred after the isolation of South America and New Zealand, geological vicariance was discarded as a possible mechanism of speciation (Crayn et al 2006).

A. chilensis is considered a “super fruit” because of its high soluble solid and antioxidant content, associated with cardioprotective, anti-inflammatory, gastroprotective, antidiabetic and antibacterial activity (Genskowsky et al 2016; Rojo et al 2012; Céspedes-Acuña et al 2018; Romanucci et al 2016), and so is the focus of increasing interest by the food industry. However, currently *A. chilensis* berries are harvested directly from the natural populations, not efficiently meeting the growing demand of this industry and which is likely contributing to the degradation and loss of genetic diversity of their natural populations (Vogel et al 2016). The harvest is usually sold fresh or as dried berries in open markets and is used to produce liquors and other products for local consumption.

Thus, there is growing interest in characterizing the genetic variation and population structure of maqui, to identify the best genotypes to use in the establishment of productive orchards with selected individuals, and eventually to register them as varieties. Recently a couple of breeding programs have been started, including one in the Universidad de Talca that has already registered a few varieties in Chile and has patented some of them in the United States of America. The most desired traits of interest include yield, berry size, seed content, and most importantly, polyphenol content and antioxidant compounds (González et al 2015). These breeding activities require germplasm from plants with the most diverse genetic background and from the widest distribution to increase the probability of assessing the greatest phenotypic diversity possible to be tested and incorporated into the new lines and varieties.

Genetic diversity studies conducted to date have included only a portion of the distribution of maqui in Chile, and have not resulted in a well-supported phylogeographic pattern. Fredes et al (2014), based on ISSR markers of 56 individuals distributed in four different provenances of central Chile (33°–39°S), found high genetic correspondence with the geographic regions sampled, identifying four main genotypes. Bastías et al (2016) described 11 SSR that were used to analyze a set of 40 individuals distributed in south-central Chile (33°S–40°S); in contrast to the previous study, they found that maqui populations had a panmictic genetic structure. More recently, Salgado et al (2017) analyzed 58 individuals from eight different provenances of south-central Chile (34°41′–41°30′S); using chloroplast microsatellite markers they were able to differentiate two main maqui haplotypes in these samples. AFLP markers were also employed, suggesting that different maqui populations were isolated by distance.

Here we assess the patterns of genetic variation of *A. chilensis* populations throughout its distribution (ca. 1,500 km in length) based on genetic variation in a newly-designed set of informative nuclear SSR

markers (Cona et al 2020), with the aim of elucidating its genetic structure and inferring the phylogeographic patterns of the species.

Materials And Methods

Sample collection and DNA extraction

Samples (between 15 and 30 at each location) of fresh young leaves of *Aristotelia chilensis* were collected between 2018 and 2019 in 14 localities from throughout the distribution of the species in Chile, prioritizing forest populations in three main areas, from north to south, central Chile, south (Araucanía) and Chilean Patagonia (Fig. 1). Quality controls reduced the total number of samples included in the study to 183 (Table 1). GPS coordinates were recorded for each sample. All samples were stored at – 80 °C in the Laboratorio de Biotecnología at the Instituto de Investigaciones Agropecuarias (INIA) in Santiago, Chile. Total genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method with 100 mg of fresh young leaves, as described (Cona et al 2020). Yield and integrity of DNA was evaluated by electrophoresis on 1% agarose stained with ethidium bromide.

Table 1

Genetic variation for microsatellites (N = 183 and 13 loci) of the *Aristotelia chilensis* samples from Chile.

Locality (abbreviation)	Geographic coordinates	N = 183	A ± SD	H _o ± SD	H _e ± SD	F _{IS}	Ne
Central Chile (CCh)		55	7.231 ± 4.285	0.516 ± 0.108	0.684 ± 0.159	0.248**	49.7
Quebrada de Macul (QM)	33°29'53.9"S, 70°30'02.2"W	15	4.769 ± 2.315	0.570 ± 0.214	0.645 ± 0.183	0.120*	38.8
Rauco (RA)	34°52'40.8"S, 71°20'42.7"W	16	5.385 ± 2.755	0.504 ± 0.214	0.619 ± 0.245	0.192***	32.0
Cerro Condell (CC)	34°59'01.5"S, 71°13'58.2"W	4	2.769 ± 0.599	0.538 ± 0.256	0.532 ± 0.201	-0.012	∞
Portezuelo (PO)	36°32'44.6"S 72°33'24.3"W	19	4.154 ± 1.864	0.481 ± 0.230	0.545 ± 0.190	0.121*	88.3
Araucanía (Ara)		61	4.538 ± 3.406	0.419 ± 0.208	0.488 ± 0.264	0.143**	∞
Villa Alegre (VA)	39°08'41"S 72°00'26"W	29	3.923 ± 2.753	0.467 ± 0.199	0.513 ± 0.238	0.092*	∞
Pichilafquen (PI)	39°13'18"S 72°15'32"W	16	3.308 ± 2.394	0.409 ± 0.251	0.472 ± 0.268	0.138*	∞
Villarrica (VR)	39°15'16"S 72°12'48"W	16	3.308 ± 1.974	0.412 ± 0.212	0.498 ± 0.253	0.178**	∞
Chilean Patagonia (ChP)		67	3.615 ± 2.022	0.325 ± 0.149	0.454 ± 0.218	0.285**	636.2
Puerto Varas (PV)	41°16'17.3"S, 72°49'31.2"W	9	2.538 ± 1.050	0.497 ± 0.231	0.507 ± 0.163	0.020	∞
Isla Tenglo (IT)	41°29'11.2"S, 72°56'57.4"W	5	2.462 ± 1.127	0.409 ± 0.359	0.589 ± 0.177	0.332*	15.9
Huelmo (HU)	41°40'14.6"S, 73°03'58.9"W	6	2.462 ± 1.050	0.386 ± 0.153	0.452 ± 0.202	0.156	∞
La Junta (LJ)	43°55'58.5"S 72°21'34.6"W	16	2.769 ± 1.787	0.450 ± 0.283	0.438 ± 0.234	-0.028	∞
Puyuhuapi (PU)	44°19'36.4"S 72°33'17.2"W	15	2.538 ± 1.050	0.296 ± 0.145	0.429 ± 0.218	0.318***	18.1

Notes: Localities are ordered from north to south. Locality groups were determined by empirical results of spatial analyses. A, average number of alleles per locus; H_e, expected heterozygosity; H_o, observed heterozygosity; SD, standard deviation; F_{IS}, inbreeding coefficient; Ne, effective population size. Asterisks denote statistical significance (*p < 0.05, **p < 0.01, ***p < 0.001),

Locality (abbreviation)	Geographic coordinates	N = 183	A ± SD	H _o ± SD	H _e ± SD	F _{IS}	Ne
Villa Mañihuales (VM)	45°10'36.3"S 72°08'25.2"W	8	1.231 ± 0.439	0.375 ± 0.171	0.530 ± 0.005	0.308	3.1
Coyhaique (CY)	45°32'08.2"S 72°04'28.6"W	8	1.462 ± 0.660	0.400 ± 0.389	0.447 ± 0.165	0.111	∞
Notes: Localities are ordered from north to south. Locality groups were determined by empirical results of spatial analyses. A, average number of alleles per locus; H _e , expected heterozygosity; H _o , observed heterozygosity; SD, standard deviation; F _{IS} , inbreeding coefficient; Ne, effective population size. Asterisks denote statistical significance (*p < 0.05, **p < 0.01, ***p < 0.001),							

Microsatellite analysis

Populations were genotyped for 13 microsatellite loci using the markers Ach3, Ach4, Ach5, Ach14, Ach28, Ach29, Ach38, Ach41, Ach45, Ach47, Ach54, Ach61 and Ach68 (Cona et al 2020). PCR amplifications contained, in a total volume of 12 µL, 3 µL gDNA (10 ng), 2.4 µL 5× colorless GoTaq Flexi buffer (Promega, Madison, Wisconsin, USA), 1.5 mM MgCl₂, 0.5 mM dNTPs (0.125 mM each), 0.3 µM primers, 0.5 U (0.1 µL) GoTaq DNA Polymerase Flexi (Promega) and completed with dH₂O. PCR conditions were the following: initial denaturation of 7 min at 94°C; then 40 cycles of 1 min at 95°C, 45 s at 58°C and 1 min 30 s at 72°C, with a final incubation of 7 min at 72°C for template filling. PCR products were separated in polyacrylamide gels (6%) and visualized using a silver-staining protocol.

We identified samples that presumably came from the same individual by searching for matching microsatellite genotypes using the Excel Microsatellite Toolkit (Park 2001) and eliminated samples if they showed more than 85% overlap. We also evaluated the existence of null alleles using the program Micro-Checker v. 2.2.3 (Van Oosterhout et al 2004). ARLEQUIN 3.5.1.2 software (Excoffier and Lischer 2010) was used to estimate allele frequency, observed heterozygosity (H_o) and expected heterozygosity (H_e). The inbreeding coefficient F_{IS} was estimated using FSTAT 2.9.4 (Goudet 2005) and Ne per population was estimated using the co-ancestry method of Nomura implemented in NeEstimator V2.1 (Do et al 2014).

Estimation and delimitation of genetic units and gene flow

We used the Bayesian clustering algorithm implemented in STRUCTURE v. 2.3.3 (Pritchard et al 2000) to group the samples genotyped with microsatellites into K clusters. We tested values of K between 1 and 23, running STRUCTURE five times for each value of K, using Evanno's ΔK method to determine the most suitable number of clusters (Evanno et al 2005). STRUCTURE was run using the admixture model and correlated allele frequencies, as recommended for populations that are likely to be similar due to migration or shared ancestry (Falush et al 2007; Pritchard et al 2000). K was estimated using 500,000 iterations, after a burn-in period of 30,000 iterations. We also estimated whether there is a

phylogeographic tendency in *A. chilensis* using analysis of molecular variance (AMOVA) on alternative population groupings in ARLEQUIN 3.5.2.2 (Excoffier and Lischer 2010).

A Bayesian analysis of population structure accounting for the geographic distribution of *A. chilensis* was performed with the R package Geneland 1.0.7 (Guillot et al 2005). The parameters for this analysis were: 1,000,000 Markov chain Monte Carlo (MCMC) iterations and 10% burn-in. To correlate genetic variation with geography, we estimated effective migration surfaces (EEMS) for microsatellite datasets (Petkova et al 2016). EEMS identifies geographic regions where genetic dissimilarity decays more quickly than expected under a null model of isolation by distance. It relates effective migration rates to expected genetic dissimilarities to identify regions of high or low migration. The program also estimates levels of effective genetic diversity across the landscape based on genetic distances between individuals sampled within a given site (deme). As such, localities with more dissimilar individuals are estimated as regions of higher genetic diversity. The EEMS method produces visualizations that highlight portions of the species' distribution where population divergence deviates from strict IBD, which in turn can be used to populations identify gene barriers and/or corridors. Divergence is represented as a function of migration rates under a stepping-stone model (Kimura and Weiss 1964). Expected genetic dissimilarities are inferred by sample locations and the migration rates within a grid of demes placed across the distribution. Individuals migrate between nearest neighbor demes with rates varying by location. For each analysis, we ran three independent chains for 10,000,000 MCMC iterations, sampling every 5,000 iterations, with a burn-in of 5,000,000 MCMC iterations, from different starting seeds and 600 demes of grid size. We compared log posterior plots to ensure convergence.

Recent migration rates among sampling locations were assessed using BAYESASS v1.3 (Wilson and Rannala 2003), an approach that does not assume H-W equilibrium. We set delta values for allele frequencies at 0.3 (maximum change between iterations), inbreeding coefficients at 0.30 and immigration rates at 0.18, so that acceptance rates for changes in these parameters fell between 40 and 60% (Wilson and Rannala 2003). BAYESASS was run three times with different random seeds to check for results convergence, with 2 million steps as burn-in and 6 million steps of data collection.

Finally, we performed a test of isolation by distance (IBD) by comparing the individual pairwise matrix of genetic distances (F_{ST}) against the Euclidean geographical distance matrix done in R3.6.1 using the Mantel test with 10,000 permutations in ARLEQUIN 3.5.2.2 (Excoffier and Lischer 2010) for alternative population groupings in ALLELES IN SPACE (Miller 2005) to assess significance.

Demographic History

To identify demographic scenarios that explain current diversity patterns in central Chile, Araucanía and Chilean Patagonia, we used the coalescent-based framework implemented in MSVAR v1.3 (Storz and Beaumont 2002) using microsatellite loci of each sampling locality. MSVAR estimates the recent effective population size (N_0), the ancestral effective population size (N_t), and the time (t) at which the effective population size changed from N_t to N_0 . Three independent runs of MSVAR were carried out including wide prior distributions of the model parameters and accounting for the possibility that the

populations remained stable over time ($N_t \sim N_0$), that there was a bottleneck ($N_t > N_0$), or a population expansion ($N_t < N_0$). Prior distributions are log-normal distribution parameterized with the mean and standard deviation for each parameter and truncated at zero following Storz and Beaumont (2002) (Table S1). Because there is no *A. chilensis* specific microsatellite mutation rate available, we used a range of mutation rates used in Elaeocarpaceae (Rossetto et al 2008), varying between 10^{-2} and 10^{-4} (Table S1). MSVAR was run for 500,000 iterations every line for 20 thousand lines under each demographic model, discarding the initial 20% of the MCMC steps as burn-in. The independent runs were used to estimate the mode of the posterior distributions of each parameter (N_0 , N_t , and t) and their corresponding 90% highest posterior density intervals. A mean generation length of three years was used to rescale the parameter t in years. Convergence of the runs was estimated with Gelman and Rubin's diagnostic using the CODA library (Plummer et al 2006) in R (R Development Core Team 2009).

Results

Genetic Diversity

The number of alleles per locus ranged from 3 to 19 in the 183 *A. chilensis* samples genotyped (Table 1), with a mean of 8.308; the mean polymorphic information content was 0.52 and the mean frequency of private alleles was 0.077. Deviation from H-W equilibrium was found due to heterozygote deficit ($P < 0.001$) in every population but Cerro Condell, Puerto Varas, Huelmo, Villa Mañihuales and Coyhaique (Chilean Patagonia). However, only three loci were not under H-W equilibrium ($P > 0.05$). Estimates of genetic diversity excluding these loci were not significantly different from those estimated with all loci, thus, we kept these markers for further analyses (Welch corrected t-test - p-value > 0.05). We found consistently moderate to high levels of genetic diversity per locality (mean expected heterozygosity ranged from 0.43 to 0.64) and high values for allele richness (mean A ranged from 1.23 to 5.38). The highest genetic diversity (Shannon diversity index) was found in Rauco (central Chile) one of the northernmost sampled populations by contrast, the southernmost populations sampled in Villa Mañihuales and Coyhaique (Chilean Patagonia) exhibited the lowest genetic diversity (Table 1). The genetic diversity values in terms of allelic richness (A) and expected (H_e) and observed (H_o) heterozygosity gradually decreased towards southern populations; the northernmost population (Quebrada de Macul) had the highest H_o (0.570) and the southernmost population of Puyuhuapi showed the lowest H_o (0.296) (Table 1).

Genetic Structure and Gene Flow

The pairwise analysis of divergence between sampling localities showed significant genetic differentiation between 96.7% of them, measured with F_{ST} (Fig. 2). Pairwise differentiation estimated with F_{ST} ranged from 0.20 to 0.56, with the largest divergence observed between Rauco and Villa Mañihuales (Table S1). The largest pairwise differentiation values were obtained between pairs of *A. chilensis* populations from the extremes of the latitudinal gradient.

The results of the STRUCTURE analysis indicated that the best clustering solution was $K = 3$ based on the ΔK method (Fig. S1), grouping *A. chilensis* population into three groups: central Chile, Araucanía and Chilean Patagonia (Fig. 3). Most of the individuals of mixed or incongruent heritage in central and austral clusters were sampled in the localities of Portezuelo, Puerto Varas and Huelmo, which except for the last are in the contact zones between groups (Fig. 2, Table 1). Consistent with this result, the variance components of the AMOVA were maximized when the populations were clustered into the same three groups ($\Phi_{CT} = 0.16260$, $P > 0.0001$; Table S2). The AMOVAs also indicated that a large percentage of the variance (72.6%) is explained by the genetic variation within populations. The genetic variation among populations within groups and between groups explained less of the variance, 11.1% and 16.3% respectively. The fixation indices indicate statistically highly significant ($P < 0.00001$) levels of genetic differentiation among populations (Table 1).

Estimates of effective migration surfaces for *A. chilensis* inferred higher than expected levels of effective migration under a model of isolation-by-distance (IBD) between Araucanía and Chilean Patagonia, but reduced migration between Quebrada de Macul-Rauco-Cerro Condell and Portezuelo in Central Chile (Fig. 4A). Congruently, the IBD analysis showed that the structure observed in the whole population of *A. chilensis* could be explained by isolation by distance (Fig. S2), supporting the presence of genetic barriers in the center and northern region of its distribution (Table S3) and one cluster in the southern region differing from the rest of the populations (Fig. 4A). In addition, the probabilities of recent migration between localities, estimated using BAYESASS, were generally low (close to 1.2%), with recent migrant individuals from central Chile to Araucanía (Table S4).

Finally, *A. chilensis* demonstrates fine-scale genetic structuring between central Chile, Araucanía and Chilean Patagonia. Estimates of effective diversity surfaces for *A. chilensis* inferred elevated diversity in central Chile but reduced genetic diversity in Araucanía and Chilean Patagonia (Fig. 4B). This is supported by estimates of expected heterozygosity that suggested that the highest genetic diversity occurs in Quebrada de Macul. However, these results could be affected by the reduced number of samples in Cerro Condell, Puerto Varas, Isla Tenglo, Huelmo, Villa Mañihuales and Coyhaique after genotype filtering. Estimates of genetic diversity for Quebrada de Macul and Rauco suggested lower than expected diversity in the two central localities (corresponding to slightly lower H_e) but did not identify any locality with elevated levels of genetic diversity (Table 1, Fig. 4B). This corresponded to the relatively even levels of H_e between sites and suggests that genomic variation is evenly partitioned across sites in this species.

Demographic History

Demographic analysis using the microsatellite data and MSVAR showed consistent results under the demographic models tested: (1) no demographic change ($N_0 = N_t$), (2) exponential change from the ancestral population size to the current population size ($N_0 > N_t$ or $N_0 < N_t$). The three independent runs for each sampling locality had Gelman and Rubin's statistics lower than 1.2. In all cases MSVAR detected evidence for major effective population size decline at all localities (Fig. 5 and Table S5). All localities

analyzed presented large ancestral effective population sizes, on the order of ~223,000 individuals (with 95% highest posterior density intervals (HPD) between ~24,000 and ~3,832,000; Table S6). Across localities the time of the bottleneck was on average ~6,400 YBP (HPD ~480 – ~71,000 YBP). Following this event, the effective population size in *A. chilensis* reached on average less than 300 (HPD ~19 – ~3,000; Table S5). However, the bottleneck events might have affected the *A. chilensis* Chilean populations in different degrees and times. While the Central population suffered the demographic events ~3,236 YBP (HPD ~269 – ~35,481 YBP), the Southern population was mostly affected ~6,474 YBP (HPD ~386–48,527 YBP). In the Patagonian population, the Villa Mañihuales and Coyhaique populations showed that bottlenecks would have affected the population long before (~12,303 YBP, HPD 851–177,828 YBP).

Discussion

We found a relatively high level of genetic diversity (GD) using a recently described set of microsatellite markers in the dioecious species *Aristotelia chilensis*, which is not unexpected for species with entomophilous pollination such as maqui (Allendes 2011). This GD level is similar to other species with similar natural history and widely distributed along the central, south and extreme south of Chile such as *Nothofagus obliqua*, *N. alpina* (Vergara et al 2014), *N. pumilio* (Mattera et al 2020), *Austrocedrus chilensis* (Arana et al 2010) and *Podocarpus nubigena* (Quiroga & Premoli 2010). The AMOVA showed that the majority of the genetic variation in *A. chilensis* occurred within populations, as expected from outcrossing species with extensive gene flow (Hamrick and Godt 1996), similar to other plant species from the South American temperate rainforest such as *Pilgerodendron uviferum* (Allnutt et al 2003), *Eucryphia cordifolia* (Segovia et al 2012), *Nothofagus spp.* (Vergara et al 2014) and *Gaultheria pumilla* (Pico-Mendoza et al 2020).

Contrary to what might be expected for a species like *A. chilensis*, which exhibits high seed dispersal by birds that consume its berries (Bravo et al 2015), the different analyses showed a clear differentiation into three groups along the latitudinal gradient: Central Chile, Araucanía and Patagonia (Fig. 3 and Table S2), refuting the hypothesis that *A. chilensis* has a panmictic genetic structure due to gene flow (Bastías et al 2016). This grouping pattern has also been reported for other species of the temperate forest in Chile and Argentina (Allnutt et al 1999, Mathiasen and Premoli 2010; Vergara et al 2014; Pico-Mendoza et al 2020) although not necessarily as coincident and differentiated as in the case described here for *A. chilensis*. This observed structure is also supported by F_{ST} values (Table S1), indicating that genetic differentiation is higher if the populations compared are more distant (Fig. 2), suggesting isolation by distance (IBD). These genetic patterns are to some extent aligned with that reported by Salgado et al (2017) for *A. chilensis* based on AFLP and chloroplast microsatellite data.

We detected a clear latitudinal pattern of north-south decrease in the level of genetic diversity in *A. chilensis*, which is coincident with a previous report by Fredes et al (2014) to help explain the variability between four *A. chilensis* populations from south-central Chile in the accumulation of anthocyanins in maqui berry fruits. A similar latitudinal trend in genetic diversity has also been reported for *Austrocedrus*

chilensis (Pastorino et al 2004, Arana et al 2010) and *Nothofagus antarctica* (Pastorino et al 2009). Interestingly, a similar pattern was also found for *Equinopsis chiloensis*, a native Cactaceae from dry central Chile (30°-34°S) (Ossa et al 2019), indicating that a xerophytic plant also experienced range contraction northwards during wet–cold conditions of the LGM, followed by expansion during the aridification of the mid-Holocene. The north-south patterns of genetic diversity observed in *A. chilensis* differ with respect to other species previously studied like the cold-tolerant *Nothofagus* (Vergara et al 2014; Ignazi et al 2019; Mattera et al 2020) and the mesothermic *Eucryphia cordifolia* (Segovia et al 2012), reflecting the different response of this plant species to the extreme climatic oscillations that occurred during the Pleistocene. During the glacial maxima great masses of ice expanded from the extreme south of South America towards more northern latitudes (Denton et al 1999), displacing the southern flora to the north (Villagrán 1990; Villagrán and Hinojosa 1997). The location of an area of high diversity of *A. chilensis* in Central Chile (Fig. 4b) suggests a great contraction of the distribution towards the north as a result of the advance of the glaciers, causing the local extinction of *A. chilensis* populations located further south and remaining in a single refuge in the central zone during LGM. During the post-glacial period, the retreat of the glaciers to the south led to recolonization as predicted by a series of founder effects in which successive population bottlenecks during range expansion produced populations with reduced genetic diversity (Hewitt 1996; Petit et al 2002). Our finding suggests that *A. chilensis* could be highly sensitive to extreme cold conditions, in comparison to the genetic diversity distribution pattern documented for cold-tolerant taxa and attributed to isolation in multiple refugia during glacial periods (Premoli et al 2000; Mathiasen and Premoli 2010; Vidal-Russell et al 2011). However, it cannot be discarded that some small populations of *A. chilensis* persisted south of this refuge in the central zone; in such a case, it is probable that these hypothetical southern populations have suffered bottlenecks, drastically reducing their genetic diversity.

Our results support the hypothesis that the post-glacial colonization of maqui occurred in the recent past from northern refugia in central Chile, with the traditionally proposed north–south colonization route (Sérsic et al 2011). The scarce presence of *A. chilensis* in the fossil pollen record makes it difficult to reconstruct the post-glacial expansion/recolonization chronology of this species towards southern Chile. Vargas-Ramírez et al (2008) detected pollen of *A. chilensis* around ~15,500 YBP in Lake Puyehue (40°41'S; northern limit of Chilean Patagonia). This implies that the climate at the time was similar to that at present, in terms of both temperature (5 to 8°C) and precipitation (Ashworth et al 1991). Under such conditions, it is probable that *A. chilensis* populations thrived in northern refugia. The demographic MSVAR analysis shows that central and Araucanía populations would have suffered bottleneck events as far as ~6,474 YBP, while this event occurred long before in Patagonian populations (i.e. ~12,303 YBP). This event might have occurred during or after the recess of ice of LGM. These breaks have been reported for other plants and animal species (Sérsic et al 2011) along the Patagonian Andes at approximately 35°S and 40.5°S, which is coincident with decreases in effective migration between central Chile, Araucanía and Chilean Patagonia.

Understanding the genetic structure and diversity of an undomesticated species is highly valuable, driving the optimization of activities such as the conservation of its diversity and the search for the locations

where the greatest genetic diversity can be found, which is useful information when domesticating a plant species for productive purposes. Maqui berry has been used as a source of pigments, medicine, food and others since pre-Colombian times by Mapuche and other native people (INIA 2012). Until recently, berries were harvested from the wild, and more recently domestication efforts and even breeding initiatives are taking place (Vogel et al 2016; Yáñez et al 2022).

Based on what has been found in this study, future explorations in search of germplasm to preserve or use the largest possible diversity might consider accessions from the northernmost limit of distribution of the species, because the greatest diversity is there. This is unexpected, considering that in the southern area of maqui distribution, berries are apparently larger and pigment content is supposed to be higher compared to northern individuals. The performance of northern material would request establishing accessions in the southern part (Araucanía for instance) and evaluating their yield and fruit characteristics. But preservation of diversity should also consider our findings, and so conservation sites (National or Regional parks and reserves) must consider the presence of maqui, which in some cases is considered a plague because of its extraordinary adaptability to harsh environments and fast growth.

Declarations

Acknowledgments: We appreciate the assistance of María Herminia Castro (INIA La Platina) for the SSRs analyses.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

PH and JCM designed the study; MC and PH collected and processed the plant material; MC completed the molecular analyses; AC and JCM analyzed the data set; MC, PL-L and PH drafted a first version of the manuscript; PL-L, JCM and PH wrote the final version of the manuscript. All authors revised and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding authors on reasonable request.

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Figures

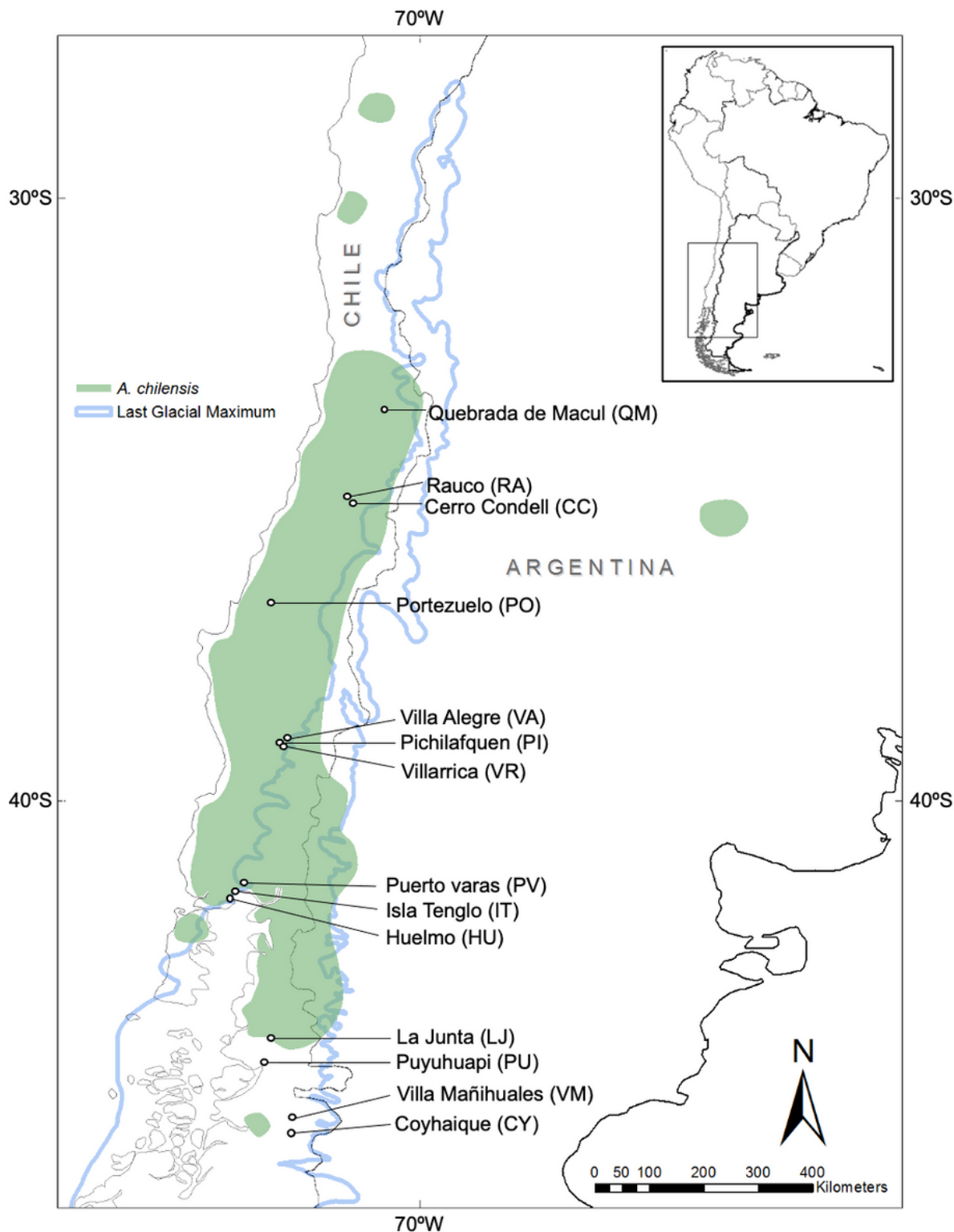


Figure 1

Map with current distribution of *Aristotelia chilensis* in Chile and Argentina. Green area represents distribution of maqui based on the Global Biodiversity Information Facility (GBIF) database considering multiple records from different herbarium datasets. Open circles represent sampled localities (see Table 1 for locality abbreviations). The solid blue line shows the limits of the ice coverage during the Last Glacial Maximum (LGM) of Pleistocene according to Hollin and Schilling (1981)

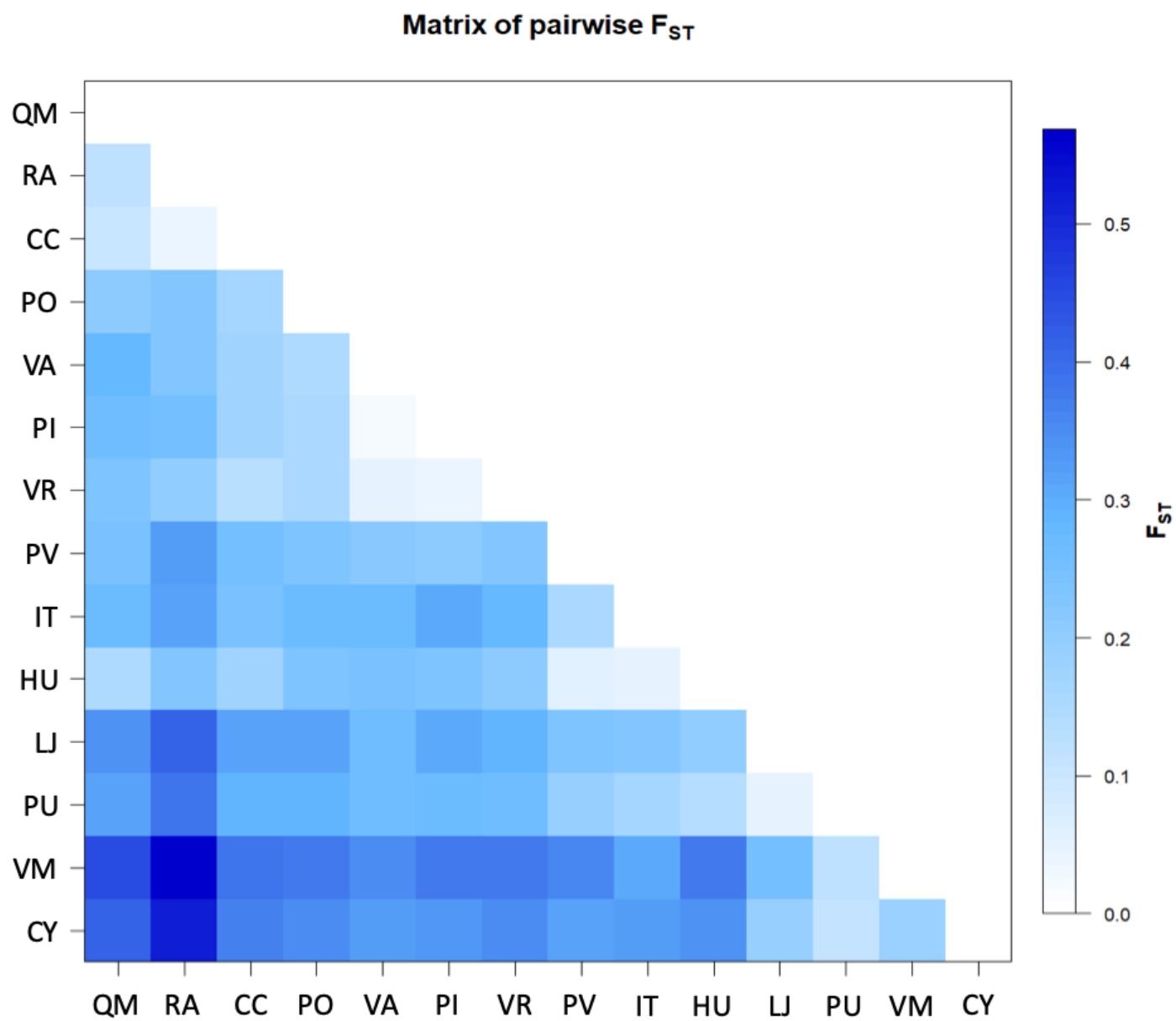


Figure 2

Plot of the pairwise F_{ST} showing comparisons between locality groups. For locality group abbreviations see Table 1

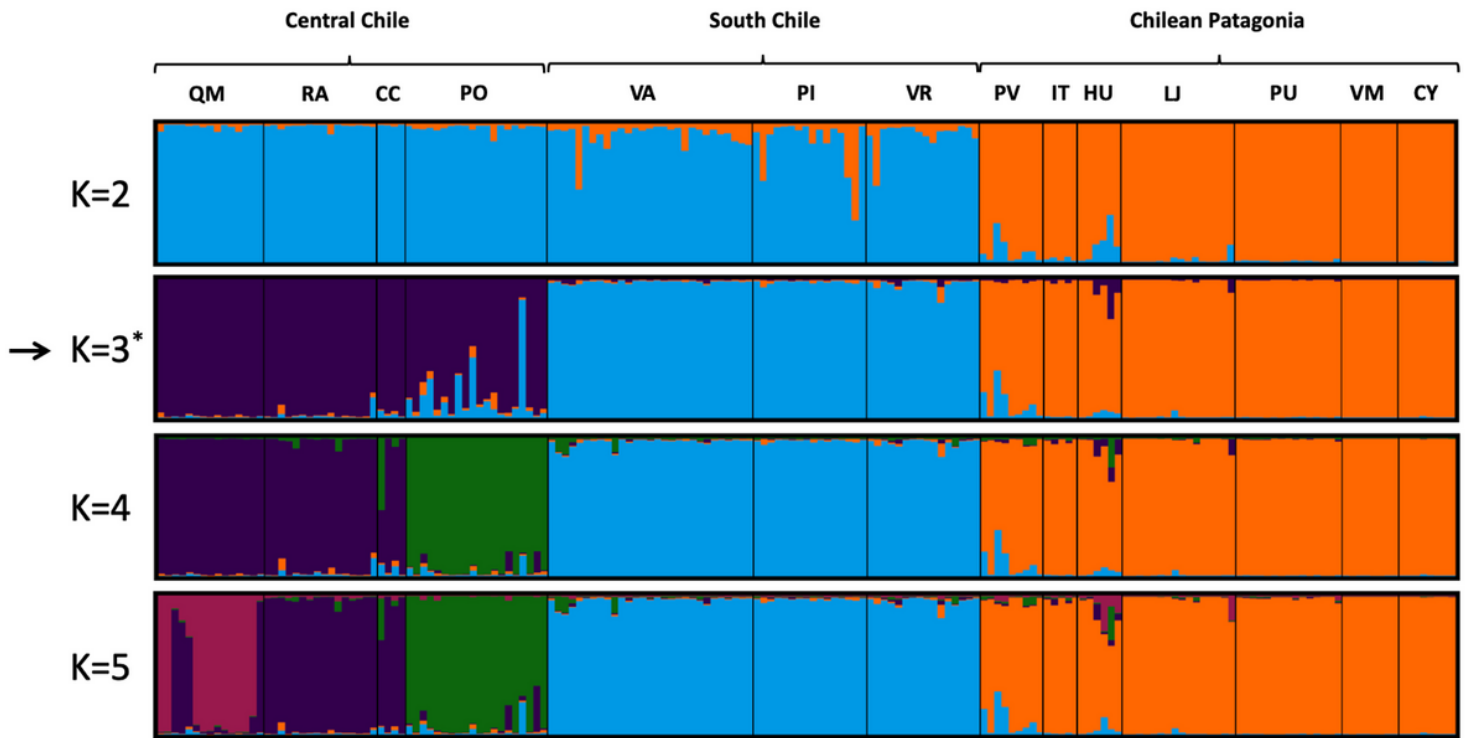


Figure 3

Plot of posterior probability of assignment for 183 plants of *Aristotelia chilensis* to three genetic clusters based on Bayesian analysis of variation at 13 microsatellite loci. Representative results from analyses of K=2, K=3, K=4, and K=5 are depicted. Dark purple = Genetic Cluster 1: Central Chile; light blue = Genetic Cluster 2: South Chile (Araucanía), and orange = Genetic Cluster 3: Chilean Patagonia. Each individual is represented with a vertical line, which are grouped by localities. Samples are arranged north to south from left to right

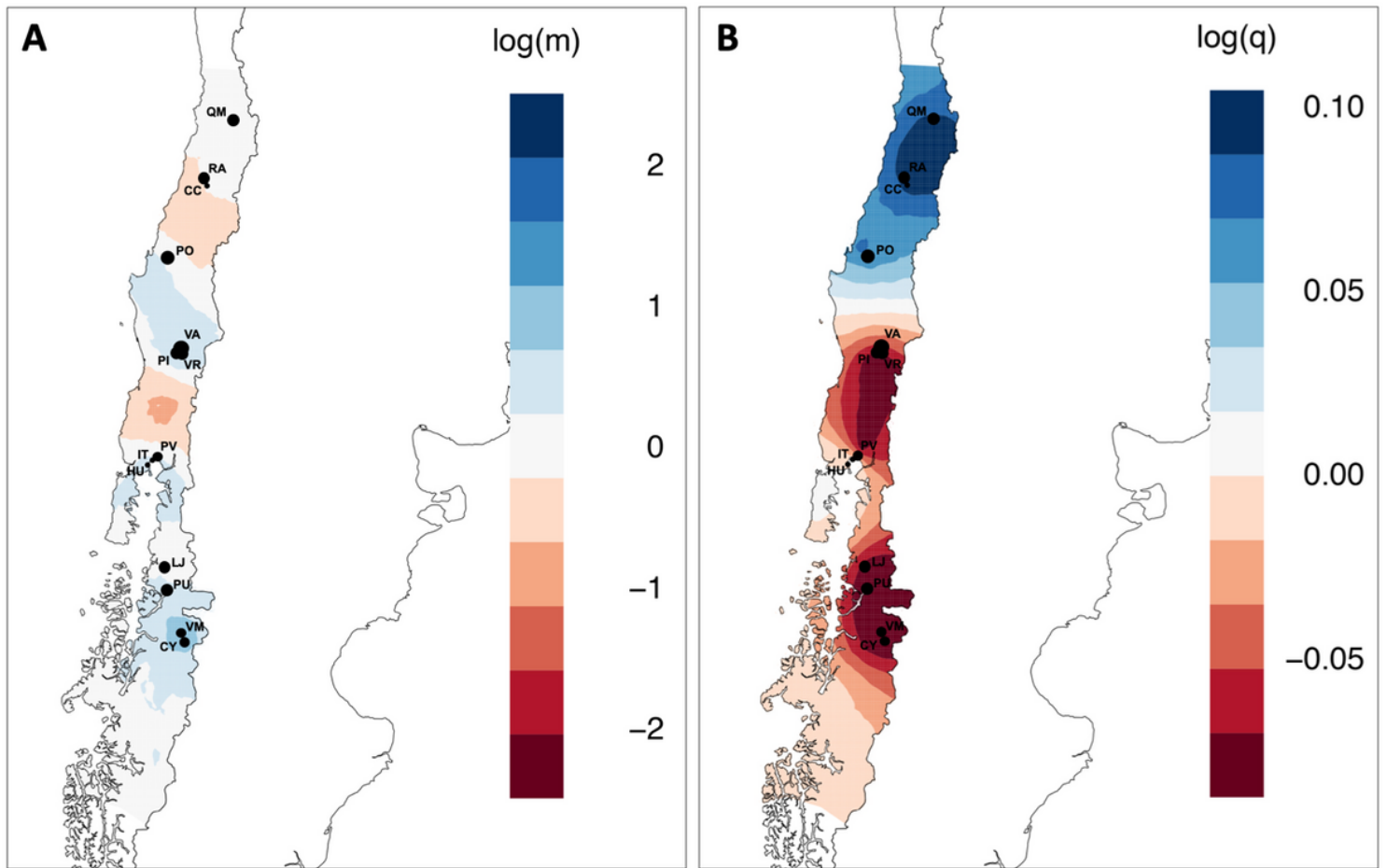


Figure 4

Estimates of effective migration (A) and diversity (B) surfaces inferred by EEMS for *Aristotelia chilensis*. (A) Blue colors represent estimated effective migration greater than that expected under isolation-by-distance, while orange colors represent barriers to movement. (B) Blue colors represent regions of elevated genetic diversity, while red colors represent regions of depressed genetic diversity. Circles indicate the sampling localities in this study.

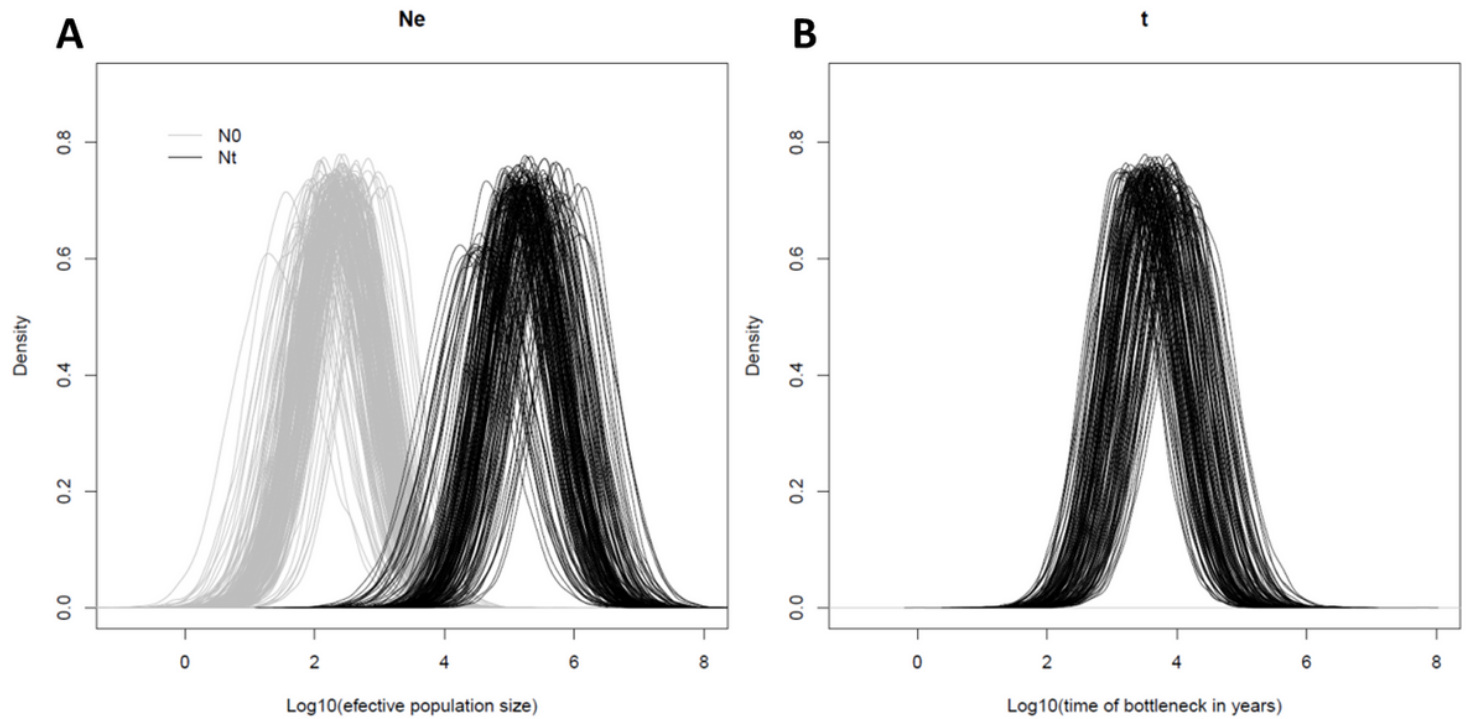


Figure 5

Demographic analysis of *Aristotelia chilensis* with MSVAR under three demographic models: population expansion, no demographic change and bottleneck. **A** shows the posterior distributions of the present effective population size (N_0) and the ancestral effective population size (N_t), while **B** shows the time when the demographic event occurred

Supplementary Files

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