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Halfway encounters: Meeting points of colonization routes among the southern beeches *Nothofagus pumilio* and *N. antarctica*

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ABSTRACT

The Patagonian region is characterized by a complex biogeographic history, with evidence of deep phylogeographic breaks shared among species. Of particular interest to conservation is the nature of colonization and settlement patterns after the last glacial period, including the detection of secondary contact between different lineages and/or hybridization among related species around phylogeographic breaks. Here we studied population demography and past hybridization of two widespread tree species endemic to South America, *Nothofagus pumilio* and *N. antarctica*.

Using 8 nuclear microsatellites we genotyped 41 populations of both species. Genetic variation and structure across the geographic region were evaluated within and among species and the past demographic history of hybridization between the two species was inferred using Approximate Bayesian Computation (ABC).

Northern and southern lineages were identified in each species, and Bayesian clustering revealed their convergence at mid latitudes (42°S). Spatial genetic structure (SGS) also indicated the existence of a genetic discontinuity at these latitudes, which is in agreement with previous data from maternal DNA markers. Several populations around 42–44°S presented high levels of genetic diversity with a decrease toward southern populations. Even though the species are clearly differentiated (G'_{ST} = 0.335), admixed gene pools were observed in both species. Two independent runs of ABC suggested that inter species admixture-like patterns occurred within the timescale of the Last Glacial Maximum (around 20,000 BP). We also provide evidences of recent and bi-directional hybridization/introgression between the two *Nothofagus* species and describe features of the populationś demography in the past. The settlement of a secondary contact zone in *Nothofagus* species around 42–44°S coincides with the phylogeographic breaks and hotspots of genetic diversity found in other plant and animal species in Patagonia, highlighting its importance as reservoir of diversity. The characterization of the population history of native species can contribute substantially to long-term conservation and management policies.

1. Introduction

The evolution of biodiversity in South America has been influenced by major large-scale geological and climatic events over millions of years. Andes uplift, tectonic movement, and Quaternary glaciations have been the dominant factors determining species diversification by creating complex geographic scenarios (Turchetto-Zolet et al., 2013 and references therein). Although knowledge about evolutionary processes in South America is increasing, the ecological survival strategies of many species of Patagonian flora and fauna and their past demographic history is still not well understood (Zemlak et al., 2008).

In the temperate Patagonian forests (36–54°S), palynological and genetic evidences for Southern beech species (*Nothofagus*) suggest either distributional shifts to northern and more favorable latitudes (Villagrán, 2001), or persistence in multiple refugia during the Last Glacial Maximum (LGM) (e.g. Azpilicueta et al., 2009; Marchelli and Gallo, 2006; Markgraf et al., 1995; Premoli

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et al., 2000). Although cold tolerant plant species, including *Nothofagus*, survived close to glaciers toward higher latitudes (54°S) (Markgraf, 1993; Mathiasen and Premoli, 2010), subsequent colonization from these multiple and distant refugia implies population bottlenecks, founding events and admixture of genetic lineages. The convergence of isolated, independently evolving, intra-specific lineages during colonization may counteract the impacts of founder events and even increase genetic diversity (Comps et al., 2001; Alberto et al., 2008; Durand et al., 2009) by the admixture of highly differentiated genetic pools (e.g. Petit et al., 2003; Vendramin et al., 1998). Thus, secondary contact zones can become important places for conservation (Grivet et al., 2008; Petit et al., 2003). Widespread, sympatric and phylogenetically related species offer good models for identifying the location of contact zones. Here, we focus on two emblematic and related species (Manos, 1997) of the Patagonian forests, *Nothofagus pumilio* and *Nothofagus antarctica*.

Phylogeographic breaks denoting isolation among genetic pools during the Quaternary and beyond have been described for different Patagonian taxa (reviewed in Sérsic et al., 2011). In particular, a deep discontinuity in maternal lineages around 42°S has been observed based on chloroplast (cp) DNA variation in both *Nothofagus pumilio* (Mathiasen and Premoli, 2010; Soliani et al., 2012) and *N. antarctica* (Acosta and Premoli, 2010; Pastorino et al., 2009; Soliani et al., 2012). These northern and southern lineages were probably isolated for a prolonged period, even before the LGM as in many other taxa from Patagonia (e.g. Cosacov et al., 2010; Mathiasen and Premoli, 2010; Sérsic et al., 2011; Soliani et al., 2012). The question remains open on how or when distant lineages met at intermediate latitudes to make up the actual configuration of continuously distributed forests. Both lineages actually met in a single population at mid latitudes (Soliani et al., 2012); therefore it would be valuable to characterize the current genetic diversity, gene flow among populations and the resulting genetic structure in the populations from these latitudes. Since dispersal ability is limited in *Nothofagus* (e.g. Donoso Zeger, 2006; Marchelli et al., 2012; Premoli and Kitzberger, 2005), it is less likely that distant southern populations (around 54°S) contributed significantly to recolonization of middle latitudes. Instead, remnant forests at 46–51°S (Glasser et al., 2008) could have been a more plausible source. On the other hand, the extensive cpDNA haplotype sharing leading to a lack of inter-species genetic differentiation between the two *Nothofagus* species suggests sympatric introgression (Acosta and Premoli, 2010; Soliani et al., 2012). Thus, the relationship between past demographic history of each species and hybridization/introgression among them still needs to be clarified.

It has been recently suggested that Quaternary glaciations would have not influenced the distribution of genetic diversity of *Nothofagus* spp., nor substantially modeled its geographic structure (Premoli et al., 2012). According to these authors, the genetic makeup of *Nothofagus* gene pools would have been modeled by more ancient geological events (backed to the Oligocene), even predating the species diversification (subgenera *Nothofagus*).

However, pollen records evidenced a forest retraction and its reduction to relicts during periods of maximum extent of ice (Iglesias et al., 2014; Quattrocchio et al., 2011; Rabassa et al., 2011; Ruzzante and Rabassa, 2011). From these relicts, reproductive success and population expansion during favorable periods would have led to the current configuration of the landscape of temperate forests including *Austrocedrus* (Arana et al., 2010; Pastorino and Gallo, 2002) and *Nothofagus* (Azpilicueta et al., 2009; Marchelli and Gallo, 2006). We expect to find here evidences of past retractions and expansions imprinted in microsatellite markers of nuclear DNA. Besides, the species must have coped with the stress associated to the great climatic changes during repeated glaciations cycles, therefore we also expect to find a higher rate of inter-specific hybridization (e.g. Heuertz et al., 2006). Altitudinal as well as latitudinal/longitudinal shifts are expected after changes in weather conditions. This may also involve alterations in individual traits related to reproduction events, like narrower temporal windows in flowers development or pollen release, with a consequent impact in fertilization processes. This may also have favored the occurrence of hybrid inter-specific individuals.

Previous studies on intra- and inter-species genetic structure in these *Nothofagus* species have been based basically on maternally inherited cpDNA which was treated as single locus (e.g. Acosta and Premoli, 2010; Soliani et al., 2012), thus multi-locus genotype data from bi-parentally inherited nuclear DNA is needed to improve our understanding of the evolutionary and demographic history of this genus. Indeed, highly variable nuclear microsatellite markers might provide accurate results about the genetic outlines described for the Patagonian region, like phylogeographic, demographic and hybridization inferences.

The main focus of this work is the identification of intra-species secondary contact between southern and northern lineages, as well as the monitoring of hybridization between the two species along a glacial transition located between 42°S and 44°S. By studying past demographic events we also intend to establish a temporal scale to the settlement of the secondary contact zone. In addition, the comparison of nuclear with previously published cpDNA data on the same set of populations (Soliani et al., 2012) would allow to discriminate between ancient and more recent processes acting at different spatial scales and to estimate rates of pollen- and seed-mediated gene dispersal. We wonder if the populations of the more recently established contact zone suffered from genetic bottlenecks during their founding. Finally, we also interpreted past demographic history of both species in order to describe the most relevant factors and temporal scales shaping the current genetic structure of populations, including frequent natural phenomena like hybridization.

Therefore, we aimed to answer the following questions: Is intra-species genetic variation geographically structured along the wide distribution range? Are there signals of admixture or secondary contact zones at mid-latitudes? If contact zones exist, when genetic lineages met? Is there any trend in the genetic diversity distribution at the nuclear genome showing signals of hybridization/introgression?

2. Material and methods

2.1. Species ecological features

Nothofagus pumilio (Poepp. & Endl.) Krasser and *N. antarctica* (G. Forster) Oerster are widely and sympatrically distributed species of the temperate forest of Patagonia in South America. They are cold tolerant species capable of plastic responses to environmental conditions, thus occurring along altitudinal, latitudinal and longitudinal gradients. *Nothofagus pumilio* forms the treeline, while *N. antarctica* shows a higher adaptive capacity growing as a tree within the forest or even as a shrub in the dry steppe. Wind dispersion of pollen and seed are shared features. The species form natural hybrids (Donoso Zeger, 2006), whose occurrence is likely due to the relative abundance of each species (Lepais et al., 2009) and the overlap of flowering times.

2.2. Plant material

A total of 41 sampled sites covering the whole natural distribution of *N. pumilio* and *N. antarctica* were included in the study. Forty populations (twenty in each species) (Table S1) from sympatric inter-specific natural forests (mixed and non-mixed,

Table 1
Analysis of molecular variance (AMOVA) between species and among populations within species for nSSRs (this study) and for cpDNA (data by Soliani et al., 2012).

Species complex	nSSRs					cpDNA				
Source	Df	SS	Est. Var	Percent	Stat	df	SS	Est. Var	Percent	Stat
Between species	1	565.4	0.818	15%	φ_{RT} = 0.15	1	2.929	0.000	0%	φ_{RT} = 0.012*
Among pops/spp.	39	990.3	0.666	12%	φ_{PR} = 0.14	38	304.897	1.023	79%	φ_{PR} = 0.29
Within pops	1278	5137.5	4.020	73%	φ_{PT} = 0.27	265	70.839	0.267	21%	φ_{PT} = 0.30
Nothofagus pumilio										
Source	*df*	SS	Est. Var	Percent	Stat	*df*	SS	Est. Var	Percent	Stat
Among Pops	19	688.3	0.892	13%	φ_{PT} = 0.13	19	134.817	0.917	81%	φ_{PT} = 0.813
Within Pops	661	3961.3	5.993	87%		131	27.667	0.211	19%	
Nothofagus antarctica										
Source	*Df*	SS	Est. Var	Percent	Stat	*df*	SS	Est. Var	Percent	Stat
Among Pops	20	540.5	0.716	12%	φ_{PT} = 0.12	19	170.081	1.127	78%	φ_{PT} = 0.928
Within Pops	617	3250.7	5.269	88%		134	43.173	0.322	22%	

df: degrees of freedom; SS: sum of squares; Est. Var: estimated variance; Stat: statistic value; Pops: populations; spp: species.
All statistic values resulted significant at $p \leqslant 0.01$except for * that is not significant (p = 1.00).

see Table 1 of Soliani et al., 2012) were sampled. Additionally, material from a single *N. antarctica* site (CHB) that showed admixture of maternal lineages was sampled (Pastorino et al., 2009; Soliani et al., 2012). A minimum of 30 individuals, at least 50 m apart, were collected from each site, resulting in 1319 samples. Greater importance was given to sampling around the possible contact zone (42–44°S), which includes a total of 21 populations.

2.3. DNA protocols

Total DNA was extracted from dormant buds following Dumolin et al. (1995). Microsatellite amplification conditions and PCR thermal profiles are described in Soliani et al. (2010). The M13 protocol (Schuelke, 2000) was applied to visualize microsatellite fragments on a MEGABACE 1000 (GE Healthcare) automatic sequencer. Seven polymorphic microsatellite loci in *N. pumilio* (*Npum*1, *Npum*3, *Npum*9, *Npum*10, *Npum*13, *Npum*17a, *Npum*18) and six in *N. antarctica* (*Npum*1, *Npum*3, *Npum*7, *Npum*9, *Npum*13, *Npum*17a) (Soliani et al., 2010) were analyzed, with 5 shared loci for the combined analyses of the two species.

2.4. Data analysis

No significant ($P < 0.05$ following Bonferroni correction in FSTAT v.2.9.3.2; Goudet, 2001) linkage disequilibrium was detected, indicating that the microsatellites used in this study are independent loci. The frequency of null alleles was estimated using FreeNA (Chapuis and Estoup, 2007) and INEST (Chybicki and Burczyk, 2009). F_{ST} was recalculated by implementing the "exclusion null alleles" (ENA) method and confidence intervals (95% level) were obtained through a bootstrap re-sampling procedure. Although a slight bias of null alleles on F_{IS} values was detected (see results), the F_{ST} corrected by null alleles was similar to the uncorrected value; therefore the original genotype data was used for further analyses.

2.4.1. Inter- and intra-species genetic diversity and population differentiation

The inter-species genetic diversity and differentiation of the two *Nothofagus* species were estimated by calculating allelic richness (A_R) after rarefaction to a common sample size (El Mousadik and Petit, 1996) in FSTAT, and the standardized differentiation value of G_{ST} index (G'_{ST}) (Hedrick, 2005) in SMOGD (Crawford, 2010). Within species genetic diversity parameters were obtained with FSTAT (N_e, effective number of alleles; A_R, allelic richness; H_O, observed and H_E, expected heterozygosity; F_{IS}, inbreeding coefficient). A Wilcoxon test for paired data (signed Rank test), i.e. population by population, using the function in R 2.13.0 (R Development Core Team, 2011) was performed to verify if the distribution of a given genetic parameter differs among species across populations.

A hierarchical analysis of molecular variance (AMOVA) was performed to evaluate the degree of genetic variation explained by (a) species, and (b) populations within species (GenAlEx 6.4. Peakall and Smouse, 2006). Statistical significance was obtained on the basis of 1000 permutations. In addition, the genetic variation between species was examined by AMOVA using published cpDNA data (Soliani et al., 2012), using the number of unshared polymorphic sites between haplotypes as genetic distance among individuals.

Significant deviations from the Hardy–Weinberg equilibrium, as indicated by deviations of F_{IS} from zero, were tested by randomization. The software BOTTLENECK (Cornuet and Luikart, 1996) was employed to identify populations experiencing a relatively recent reduction in effective population size, using the Stepwise Mutation Model (SMM), Infinite Alleles Mutation Model (IAM) and Two-Phased Mutation Model (TPM), the latter being the recommended model. Departures from the mutation-drift equilibrium were tested using the Wilcoxon signed rank test.

2.4.2. Spatial genetic structure (SGS) and its heterogeneity between genomes and species

A spatial genetics correlation test was used to evaluate a correspondence between genetic variation and the geographical layout of genetic variants in populations. Due to the vast extension of *Nothofagus* forest in their natural distribution, with a changing topography associated mainly to latitude, it may be of great importance to evaluate the impact of extrinsic factors over alleles/haplotypes allocation among populations and to identify putative barriers to gene flow.

For SGS analysis, multi-locus genotypic distances between individuals were calculated and then spatial autocorrelation coefficients, *r* (Smouse and Peakall, 1999), were obtained for ten 200 km distance classes, using GenAlEx 6.4 (Peakall and Smouse, 2006). The upper and lower confidence limits for the 95% confidence interval based on the null hypothesis of no spatial structure, and the autocorrelation of *r*, were tested for statistical significance by 1000 permutations. Upper and lower error limits for the 95% confidence interval were determined by re-sampling 1000 bootstrap values. The SGS analysis was applied also to cpDNA data from Soliani et al. (2012), which considers unshared polymorphic sites as genetic distance between individuals (or haplotypes). To analyze the heterogeneity of the SGS among distance classes and datasets, we tested each genome (cpDNA and nSSRs) by comparing paired

species (*N. pumilio* and *N. antarctica*), based on the method described by Smouse et al. (2008). Fisher's combined probability criterion (ω) was applied to test for the departure of the entire correlogram from the null hypothesis of no spatial structure, and values for each distance class were computed using the tail probabilities (*P*-values). The heterogeneity of the entire correlogram for each of the four datasets was evaluated (Smouse et al., 2008), employing single-distance (t^2) and multi-distance class criteria (ω). The significances of both criteria were determined on the basis of 1000 permutations using GenAlEx 6.4 (Peakall and Smouse, 2006).

2.4.3. Individual-based genetic structure and admixtures

To evaluate genetic and geographic structure and admixture within and between species, a Bayesian cluster analysis using STRUCTURE (Pritchard et al., 2000) was performed, based on the LOCPRIOR model (Hubisz et al., 2009), with admixture and correlated allele frequencies (hereafter, the *F*-model) as described by Falush et al. (2003). The shared polymorphic loci were examined on all 41 populations, resulting in a total of 1279 individuals after the exclusion of the samples with missing data at more than two loci. The analysis was also conducted at the intra-species level, employing 7 and 6 loci for *N. pumilio* and *N. antarctica*, respectively. Three independent runs for each *K* (from 1 to 15) were performed with a 100,000 burn-in period and 500,000 repetitions and the optimal number of clusters was evaluated based on the rate of change in the log probability of data between successive *K* values (ΔK) (Evanno et al., 2005). Membership coefficients to each inferred cluster were post-processed using CLUMPP (Jakobsson and Rosenberg, 2007) and edited with DISTRUCT (Rosenberg, 2004).

2.4.4. Demographic inference of gene exchange between the species

The demographic history of hybridization among the two species was analyzed employing Approximate Bayesian Computation (ABC) in DIYABC v1.0.4.39 (Cornuet et al., 2010, 2008) using the dataset of the 5 common loci. DIYABC provides flexibility for the mutation models of microsatellite loci in coalescent simulations (e.g. generalized stepwise mutation model, single nucleotide indel). However, as DIYABC does not assume gene flow after the divergence and/or admixture, and considering that low intra-species population differentiation was found in this study, ABC analysis was only performed at the inter-species level, where clear and significant genetic differentiation was detected. Since STRUCTURE showed an admixture-like pattern in both species (see results), the analyses were subdivided into '*Inter-species admixture in N. pumilio populations*' (ABC1) and '*Inter-species admixture in N. antarctica populations*' (ABC2). To simplify demographic scenarios, three populations were defined in each ABC analysis, according to the results by STRUCTURE analysis. An additional criterion that was considered for population choice is their geographic proximity. Populations showing a high proportion of co-ancestry of the parental species and admixed populations were selected as follows:

ABC1: Pop1 (corresponding to pop 12 of "pure" *N. pumilio*), Pop2 (corresponding to pops 8 and 9 of *N. pumilio* showing admixture), and Pop3 (corresponding to Pops *VII* and *X* of "pure" *N. antarctica*).

ABC2: Pop1 (corresponding to Pops 7 and 10 of "pure" *N. pumilio*), Pop2 (corresponding to pops *VIII* and *IX* of *N. antarctica* showing admixture), and Pop3 (corresponding to Pops *VII* and *X* of "pure" *N. antarctica*).

In the four scenarios examined (Fig. 4), t# means the time scale measured by generation time, and N# the effective population size of the corresponding populations (e.g. Pop1, Pop2, Pop3, ancestral population "a") during the time period (e.g. 0–t1, t1–t2).

Scenario 1 – *isolation with admixture model*: since Pop2 is the putative hybrid, it was set as generated by admixture of Pop1 and Pop3 at t1. Then, Pop3 merged with Pop1 at t2.

Scenario 2 – divergence at the same time from a common ancestral population: all 3 populations diverged at t2.

Scenario 3 – *hierarchical divergence from an ancestral population, case 1*: Pop2 merged with Pop1 at t1 and then, Pop3 with Pop1 at t2.

Scenario 4 – *hierarchical divergence from an ancestral population, case 2*: Pop2 merged with Pop3 at t1 and then, Pop1 with Pop3 at t2.

In all scenarios, since we assumed population expansion after the divergence, the effective population size of the ancestral population, "Na", before t2, was set as smaller than the other populations (e.g. Pop3) after t2. In addition, on the basis of the distribution of the genetic diversity across the selected populations, the effective population sizes were set as follows: N2 > N1 and N3 $\geqslant$ N2. The higher mutation rate in the generalized stepwise mutation model (GSM) (Estoup et al., 2002) with the lower mutation rate of single nucleotide indels (SNI) were employed for modeling mutation of SSRs. The default prior values were used for all these parameters (Table S2). The mean values of expected heterozygosity (H_E) and the number of alleles (N_A) were used for the summary statistics of each of the three populations. Additionally, for each population pair, genotype likelihood and F_{ST} were used. A million simulations were performed for each scenario. After a total of four million simulations, the most-likely scenario was evaluated by comparing the posterior probabilities by the logistic regression method. The goodness of fit of the scenario was assessed by the option "model checking" with principal component analysis (PCA), which measures the discrepancy between model and real data.

3. Results

3.1. Inter specific genetic variation

More than one hundred alleles (126) were identified, the majority of which shared among species. Private alleles were mostly found at intermediate latitudes (40–44°S): thirteen for *N. pumilio* and only five for *N. antarctica*.

The differentiation values with (F_{STENA} = 0.138[0.048–0.241]) and without implementing ENA correction (F_{ST} = 0.145[0.053–0.254]) did not differ substantially, and were both significantly different than 0 after 1000 bootstrap re-sampling over loci (CI 95%). According to the Wilcoxon paired test, all the genetic diversity parameters of *N. antarctica* were significantly higher than in *N. pumilio* (N_e = 3.55 *vs.* N_e = 3.16, p = 0.022; A_R = 4.80 *vs.* A_R = 4.45, p = 0.015; H_E = 0.656 *vs.* H_E = 0.598, p = 0.029; H_O = 0.520 *vs.* H_O = 0.450, p = 0.017). The hierarchical AMOVA showed that the proportion of genetic variance partitioned between the two species was 15% for nSSRs (φ_{RT} = 0.15, p = 0.001) while less than 1% for cpDNA (φ_{RT} = 0.012, p = 1, Table 1). Standardized genetic differentiation between the species was also higher for nSSRs (G'_{ST} = 0.335) than for cpDNA (G'_{ST} = 0.061) data.

3.2. Intra-species genetic variation

Higher allelic richness and gene diversity values were found in *N. pumilio* populations located around 37–42°S (populations 2, 3, 4, 6 and 8), whereas at about 40–43°S (*V*, 00, *VIII* and *IX*) in *N. antarctica* (Table 2). A decrease in A_R and H_E toward southern populations (beyond 42°S) was more evident in *N. antarctica* than in *N. pumilio*, in agreement with previous results based on cpDNA data (Soliani et al., 2012). Wilcoxon signed rank test for bottlenecks was significant (p = 0.05) for both the TPM and IAM models in the

Table 2
Genetic diversity parameters estimated at the population level for both species.

R	P	Lat (S)	*Nothofagus pumilio*									*Nothofagus antarctica*								
			ID	N	N_e	H_O	H_E	H_E*null*	F_{IS}	A_R (*g*=13) [7loci]	A_R (*g*=9) *[5loci]	*ID*	N	N_e	H_O	H_E	H_E*null*	F_{IS}	A_R (*g*=8) [6loci]	A_R (*g*=9) *[5loci]
North	E	36°	*1*	32	3.9	0.543	0.688	0.709	0.0004	5.6	4.0	*I*	34	3.9	0.533	0.685	0.694	0.000	5.0	4.4
	CAV	37°	*2*	26	4.0	0.556	0.662	0.672	0.000	6.2	4.7	*II*	30	4.3	0.460	0.686	0.722	0.025	5.5	5.0
	Tr	39°	*3*	34	5.0	0.543	0.708	0.730	0.0001	6.9	5.2	*III*	30	3.7	0.439	0.695	0.735	0.001	4.8	4.8
	Q	40°	*4*	29	4.2	0.497	0.649	0.690	0.001	6.3	4.6	*VI*	32	4.9	0.564	0.740	0.771	0.000	5.7	5.2
	V	41°	*5*	29	4.4	0.399	0.681	0.712	0.000	5.8	4.1	*V*	28	5.5	0.374	0.782	0.771	0.000	5.9	5.3
Centre	CHB	–			–	–	–	–		–		00	29	5.5	0.528	0.794	0.792	0.000	6.1	5.3
	Hm	42°	*6*	60	4.5	0.474	0.678	0.730	0.0735	6.4	4.4	*VI*	30	3.9	0.577	0.712	0.751	0.000	4.9	4.5
	H	42°	*7*	31	3.3	0.509	0.634	0.674	0.000	5.2	3.9	*VII*	31	3.8	0.615	0.704	0.737	0.000	4.9	4.6
	Np	42°	*8*	29	4.6	0.373	0.704	0.754	0.157	6.2	4.4	*VIII*	32	4.6	0.672	0.692	0.708	0.000	5.4	5.2
	Te	43°	*9*	30	3.7	0.497	0.704	0.732	0.000	6.0	4.8	*IX*	30	4.8	0.570	0.746	0.775	0.099	5.8	5.3
	Eg	43°	*10*	43	3.9	0.412	0.578	0.607	0.002	5.4	3.6	*X*	30	4.1	0.517	0.723	0.763	0.106	5.2	4.7
	G	43°	*11*	62	4.2	0.481	0.653	0.692	0.000	6.1	4.3	*XI*	28	4.2	0.599	0.680	0.721	0.0005	5.3	4.9
	JSM	43°	*12*	32	2.9	0.390	0.548	0.599	0.000	4.9	3.8	*XII*	30	3.7	0.530	0.691	0.743	0.000	4.8	4.6
	F	44°	*13*	32	4.2	0.491	0.633	0.688	0.142	5.7	4.4	*XIII*	32	3.1	0.569	0.633	0.666	0.000	4.6	4.3
	U	44°	*14*	30	3.8	0.458	0.664	0.708	0.0008	5.6	4.3	*XIV*	30	3.3	0.507	0.641	0.699	0.000	4.6	4.3
	AP	44°	*15*	32	3.1	0.341	0.545	0.591	0.0002	4.8	3.3	*XV*	32	4.4	0.581	0.642	0.675	0.000	5.4	4.6
South	CC	51°	*16*	29	3.7	0.506	0.639	0.675	0.000	5.4	4.4	*XVI*	29	3.5	0.575	0.677	0.713	0.000	4.5	3.2
	MI	51°	*17*	30	3.9	0.563	0.631	0.639	0.000	5.7	4.3	*XVII*	30	2.9	0.477	0.546	0.584	0.000	3.8	3.9
	TdFN	54°	*18*	30	3.8	0.443	0.666	0.719	0.0001	5.7	4.1	*XVIII*	31	3.6	0.489	0.655	0.705	0.073	4.9	4.6
	TdFE	54°	*19*	29	3.6	0.549	0.645	0.677	0.000	5.5	4.2	*XIX*	29	4.4	0.484	0.690	0.727	0.012	5.3	4.7
	TdFC	54°	*20*	32	3.9	0.521	0.699	0.735	0.000	5.9	4.7	*XX*	31	3.5	0.595	0.663	0.696	0.000	4.9	4.6

R: geographical region; P: population acronym from Table S1; Lat (S): south latitude; N: sample size; N_e: effective number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; H_E*null*: corrected H_E considering null alleles; F_{IS}: inbreeding coefficient; A_R: allelic richness with *g* = rarefaction number. *Allelic richness was obtained based on common loci and with rarefaction to a common sample size.

following populations: *1* and *5* for *N. pumilio* and *IV*, *V*, 00, *IX*, *XII* and *XIX* for *N. antarctica*).

Four populations of *N. pumilio* (*6*, *8*, *10* and *13*) from the central region and three of *N. antarctica* (*IX*, *X* and *XVIII*) from central or southern regions showed signs of inbreeding after excluding the bias introduced by null alleles using INEST (Table 2). Jackknifing across loci was used to calculate standard errors of the estimates, revealing the absence of significance (i.e. $F_{IS} = 0$) of inbreeding coefficients in each tested population.

Genetic differentiation was significant in both taxa and slightly higher in *N. pumilio* ($F_{STN.pumilio} = 0.094$, $p = 0.001$, and $F_{STN.antarctica} = 0.083$, $p = 0.001$). The standardized differentiation (G'_{ST}) was higher but similar in both species ($G'_{STN.pumilio} = 0.296$ and $G'_{STN.antarctica} = 0.303$).

3.3. Spatial genetic structure and its heterogeneity between species

Spatial autocorrelations were stronger in cpDNA than in nSSRs for both species, although all datasets displayed significant positive spatial autocorrelations at distance classes up to 400 km (Fig. 1, Table 3). Both the *single-distance class* (t^2) and the *multi-distance class criteria* (ω) showed significant differences among species and genomes at all distance classes ($P < 0.01$), with some exceptions (Table 3, Fig. 1). In cpDNA, both species presented significant positive autocorrelations at larger distance classes (1200 and 1400 km). A similar pattern was observed with nSSRs of *N. pumilio* at 1600 km. At the intra-specific level, only *N. antarctica* showed a pattern of isolation by distance (Fig. S4).

3.4. Individual-based genetic structure and admixtures

In the STRUCTURE analysis of the 41 populations, ΔK indicated that the optimal number of groups was $K = 2$, with a clear separation between the species (Fig. 2). Some populations presented a high level of admixture, suggesting hybridization and even introgression through backcrosses, e.g. populations *8* and *9* in *N. pumilio*, and *VIII, IX, XII* and *XIII* in *N. antarctica*. Evidence of inter-specific gene flow was also observed when cluster partitioning was increased (for example at $K = 4$ and $K = 5$, Fig. 4). But, Bayesian clustering might also be interpreted as the consequence of demographic variation among populations of the same species. In order to acknowledge this question we used ABC analysis, having in mind the amount of genetic diversity and pairwise differentiation for pure and admixed selected clusters included in the analysis. A significant amount of pairwise genetic differentiation among clusters was found (ABC1 and ABC2, Table 4). Also, genetic diversity was highest in the *N. antarctica* cluster, and intermediate in the admixed cluster (Table 4).

At the intra-specific level, a geographical trend depicting a cline for genetic variation was found. Within each species, the coexistence of two main groups, northern and southern, was identified at intermediate latitudes (suggested contact zone) (Fig. 3). In addition, none of the clusters were shared among edge distributed populations (i.e. north and south) (see *kriging* implementation and results in Fig. S1). The highest values of ΔK were detected at $K = 4$ in *N. pumilio* (Fig. 3A) and at $K = 3$ in *N. antarctica* (Fig. 3B). In both species, Challhuaco (*5*, *V*) and R. Unión (*14*, *XIV*) were identified as putative admixed gene pools. But, additional species-specific sites were also recognized, e.g. *6* and *10* (*N. pumilio*) and *VIII* and *XII* (*N. antarctica*).

3.5. Demographic models tested with ABC

In ABC1, the highest posterior probability was found for scenario 1 (0.7696, isolation with admixture model, Fig. 4) and its value was much higher than the other scenarios (0.0475–0.1346, Table 4). The median values for the time of the admixture at t1 and the divergence at t2 were estimated to be 379 and 6050 generations ago, respectively (IC 95% are reported in Table S3). Similar admixture rates, *ra*, were detected between each pure and the admixed population (Pop3–Pop2 = 0.436 and Pop1–Pop2 = 0.564). All estimated parameters (effective population size, mean

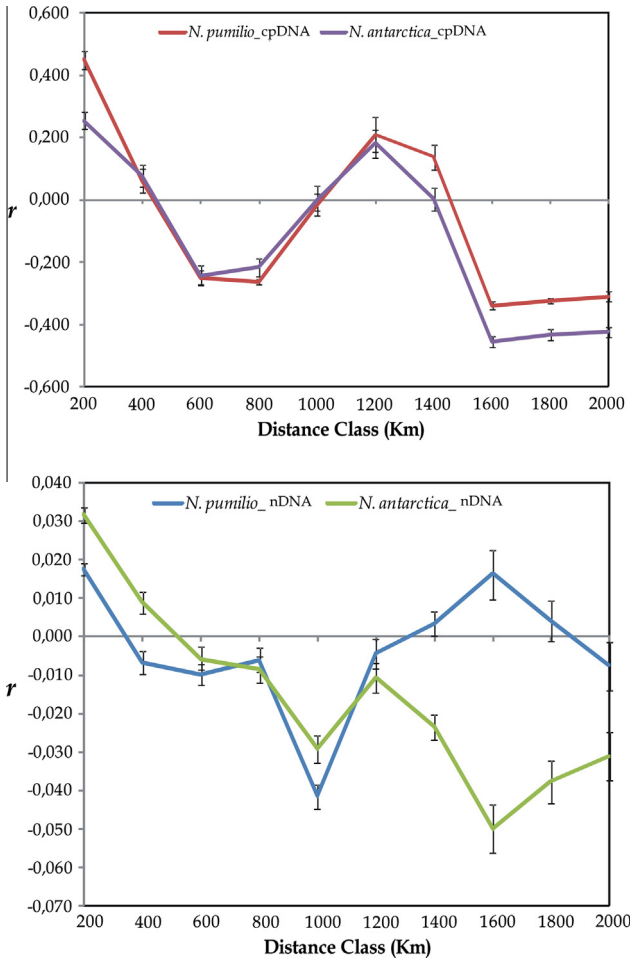

Fig. 1. Spatial genetic structure analysis (single and multi-distance class) estimated by the heterogeneity test of nSSRs and cpDNA data (Soliani et al., 2012) in each species.

mutation rates of SSR, multiple stepwise mutations *P*, and SNI) are shown in Table S3. The distributions of the posteriors suggested that N2, N3, Na and t2 were poorly estimated (Fig. S2). All of the 21 summary statistics (values) in each population and for all possible combinations of population-pairs (simulated using obtained posterior distribution of each parameter) were not significantly different from the observed values (Table S4). In the PCA, a large cloud of data simulated from the prior and the observed data set was observed in the middle of a small cluster of datasets from the posterior predictive distribution (Fig. S3). These results suggested a good fit of the scenario to the observed data (Table S4).

In ABC2, the most-likely scenario was number 4 (hierarchical divergence from an ancestral population) having the highest posterior probability (0.6313, Fig. 4, Table 4). The median values of the divergence time were estimated as t1 = 890 (split between Pop 2 and Pop3) and t2 = 6210 (split of Pop1 from the others) generations ago. All estimated parameters are shown in Table S5. The distribution of the posteriors indicated that N3, Na and t2 were poorly estimated (Fig. S2). As in ABC1, the summary statistics showed that all the observed values were not significantly different from the simulated values (scenario 4), thus suggesting a good-fit of the scenario to the observed data (Fig. S3, Table S6).

According to the species ecological traits and to Veblen et al. (1996), generation times of 50 years for *N. pumilio* and 30 years for *N. antarctica* were assumed. Thus, the admixture time in ABC1 is 18,950 years and the divergence time 302,500 years. In the case of ABC2, the divergence times were 26,700 and 186,300 years.

4. Discussion

4.1. Evolutionary history of Nothofagus

Comparative phylogeography of Patagonian flora and fauna suggests that Pleistocene climatic oscillations and Pliocene/Miocene orogenic events have contributed to shaping the current distribution of modern lineages (Sérsic et al., 2011; Turchetto-Zolet et al., 2013). Although previous studies have suggested an impact of ancient geological events upon the genetic structure of *Nothofagus* spp. (e.g. Premoli et al., 2012), our results showed inter-species admixture by the time of the LGM (around 20,000 BP) and settlement of a contact zone after recolonization. The employment of nuclear microsatellites, the markers with high mutation rates in the bi-parental inherited genome, allowed the detection of the recent dynamics of population demography in this study.

Topographic barriers might have imposed restrictions to gene flow among populations i.e. marine introgressions, ice sheets, volcanic arches, Andes orogeny or river basins (Cosacov et al., 2010; Premoli et al., 2012; Rabassa et al., 2005). Large Patagonian rivers like the Chubut and the Deseado might also have presented impassable barriers to migration and dispersal for terrestrial flora and fauna (e.g. Morando et al., 2007; Martínez and Coronato, 2008; Sede et al., 2012; Cosacov et al., 2012). Depending on life history traits of each particular species (e.g. dispersal ability, Duminil et al., 2007; Hamrick and Godt, 1996) alternative strategies to climatic changes were necessary, e.g. long term *in-situ* persistence (*Mullinum* spp., Sede et al., 2012), fragmentation, recruitment and/or population expansion (*Nothofagus* spp., Azpilicueta et al., 2009; Marchelli and Gallo, 2006). For the broadly distributed *Nothofagus* species, a phylogeographic break around 42°S explained the genetic structure of their populations (Mathiasen and Premoli, 2010; Pastorino et al., 2009; Soliani et al., 2012). Our results with highly variable nuclear markers confirmed the presence of this break. The spatial genetic structure (SGS) shows the break in the two species, leading to a coherent trend both at

Table 3
Heterogeneity tests of spatial genetic structure among molecular markers (nSSRs and cpDNA) and species (*Nothofagus pumilio* and *N. antarctica*), using single-distance class criteria (t2) and multi-distance class criteria (ω).

Species/genome pair	t^2 criteria for each distance class interval (km)										ω criteria
	0–200	201–400	401–600	601–800	801–1000	1001–1200	1201–1400	1401–1600	1601–1800	1801–2000	–
nSSRs$_{pumilio}$ vs. nSSRs$_{antarctica}$	93.436	51.950	3.212	1.154	24.790	4.596	124.505	166.939	99.739	25.784	111.686
P-value	0.001	0.001	0.074	0.280	0.001	0.027	0.001	0.001	0.001	0.001	0.001
cpDNA$_{pumilio}$ vs. cpDNA$_{antarctica}$	650.948	4.21	0.268	21.434	2.639	5.076	159.107	33.847	31.53	27.239	102.504
P-value	0.001	0.044	0.609	0.001	0.098	0.021	0.001	0.001	0.001	0.001	0.001

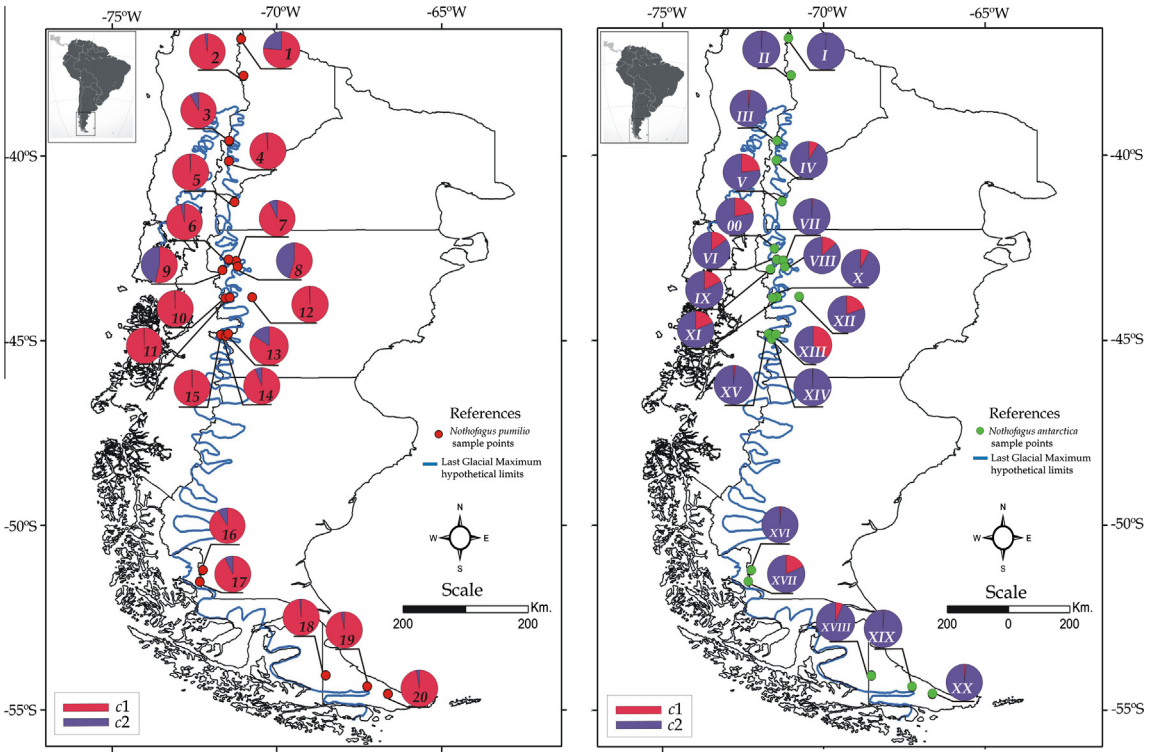

Fig. 2. Bayesian clustering (using STRUCTURE, Pritchard et al., 2000) of *N. pumilio* and *N. antarctica* populations. Pie-charts represent population membership coefficients (pop-*Q*) at the optimum grouping (*K* = 2). *c*1: *pumilio* cluster; *c*2: *antarctica* cluster. Last Glacial Maximum (LGM) advanced is indicated based on (Glasser et al., 2008).

Table 4
Posterior probability of each tested scenario and its 95% confidence interval based on the logistic estimate according to DIYABC. H_S: genetic diversity and G'_{ST}: standardized genetic differentiation in the examined populations.

Hs	G'_{ST}		Scenario	Posterior probability	95% CI (lower–upper)
Pop1 = 0.531	Pop1–Pop2 = 0.26 (p = 0.001)	ABC1	1	0.7696	0.7476–0.7916
Pop2 = 0.699	Pop1–Pop3 = 0.584 (p = 0.001)		2	0.0483	0.0404–0.0562
Pop3 = 0.713	Pop2–Pop3 = 0.277 (p = 0.001)		3	0.1346	0.1168–0.1524
			4	0.0475	0.0393–0.0558
Pop1 = 0.543	Pop1–Pop2 = 0.467 (p < 0.05)	ABC2	1	0.2545	0.2374–0.2716
Pop2 = 0.705	Pop1–Pop3 = 0.524 (p < 0.05)		2	0.0915	0.0826–0.1004
Pop3 = 0.713	Pop2–Pop3 = 0.20 (p < 0.05)		3	0.0224	0.0187–0.0261
			4	0.6316	0.6119–0.6513

organelle and nuclear genomes. Indeed, additional analyses did not reveal structure within northern and southern groups, suggesting that SGS was generated by barriers to gene flow along the contact zone, either ice-sheet during the LGM or topography. North–south orientated mountain chains parallel to the Andes at 42–44°S led to the advance of irregular eastwards ice-lobes shaping a singular frozen landscape (Flint and Fidalgo, 1969). Then, the negative autocorrelation at shorter distance classes could have been due to the combination of highly differentiated populations along short geographic distances close to the barrier. Gene flow among these populations could be restricted depending on their location within the same or neighboring hills and their exposure to the prevailing west-east winds. It might be hypothesized that remnant forests remained isolated (maybe fragmented), until a new wave of inter-population gene flow increased genetic diversity via lineage admixture (Alberto et al., 2008; Durand et al., 2009) and caused the settlement of secondary contact zones at the end of unfavorable periods through population expansion.

The 42°S-break was recently interpreted as a long-lasting isolation of ancient lineages that dates back to the Paleogene, meaning the persistence over time of ancient polymorphisms (Premoli et al., 2012). The authors explicitly sustain that the genetic structure of *Nothofagus* "was not reset by Quaternary glaciations". However, the assumed ancient tectonic control of vegetation and species gene pools fails in explaining the current patterns of variation, where no genetic structure was found with isozymes (Mathiasen and Premoli, 2010). The lack of coherence between maternal (Acosta and Premoli, 2010; Mathiasen and Premoli, 2010) and bi-parental inherited markers (Mathiasen and Premoli, 2010), with opposite trends in genetic diversity, would reduce, at least in part, the strength of the palaeogeological theory.

Certainly, Paleogene events might have impose a stamp in the genetic structure of existing taxa, but the impact of Quaternary glaciations should not be disrespected. The finding of intra-population variation at cpDNA and lineage admixture in the suture zone highlights the impact of recent past climate changes. A single population of *N. antarctica* (i.e. Cholila, 42°31′32′S–71°31′34″W) showed intra-specific admixture with cpDNA (Soliani et al., 2012), and now nuclear SSRs additionally support the hypothesis of a recent establishment of the contact zone. As a result, nuclear clusters that characterized northern and southern populations converge in the same area (Fig. 3).

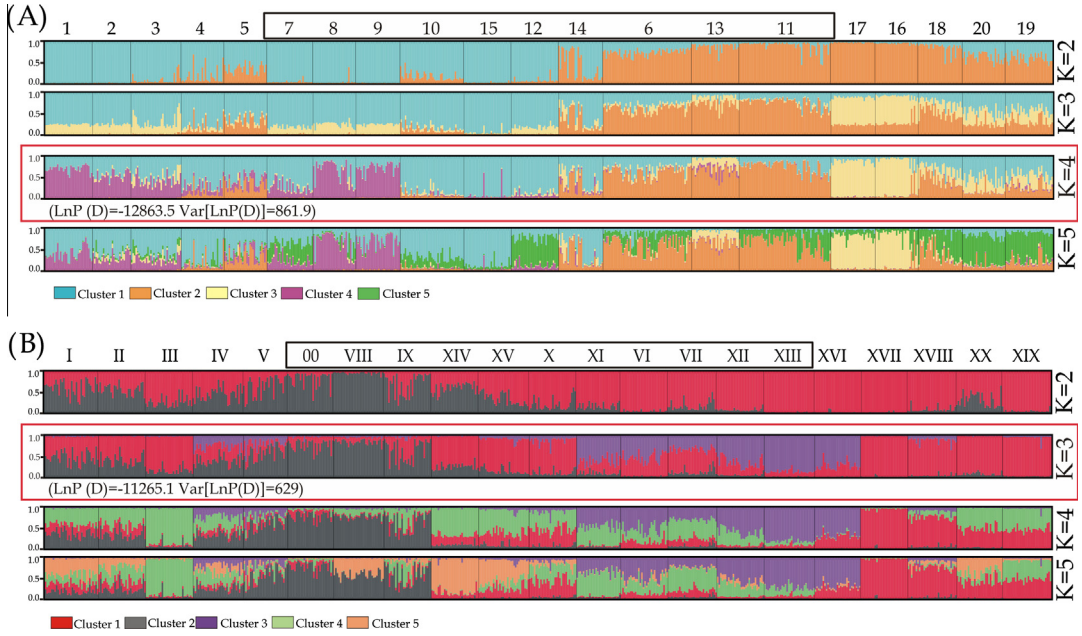

Fig. 3. Individual membership coefficients for genetic demes of *Nothofagus* species ($K = 2$ to $K = 5$, STRUCTURE, Pritchard et al., 2000). (A) *Nothofagus pumilio*, (B) *N. antarctica*. Colors represent clusters being independent among species. Black box: populations localized at the putative contact zone. Red boxes: optimum grouping as inferred in (Evanno et al., 2005). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

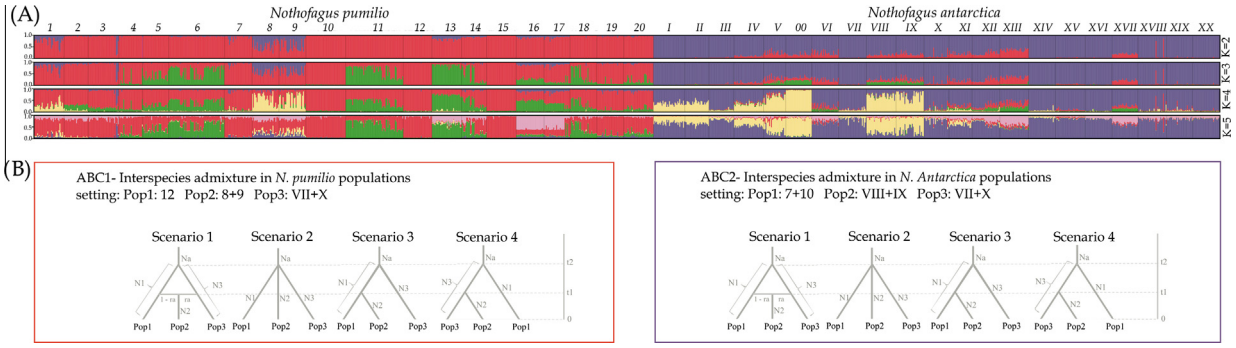

Fig. 4. Inferred genetic structure at the between species level ($K = 2$ to $K = 5$, STRUCTURE, Pritchard et al., 2000) (A) and past demography scenarios tested with DIYABC (B).

Actually, alternative non-exclusive hypotheses to explain the genetic structure of these populations cannot be discarded, owing to the particular topography of Patagonia and glacial patterns associated (Flint and Fidalgo, 1964, 1969; Rabassa et al., 2005). First, northern populations found more favorable slope-down habitats (ice-free areas) (Villagrán, 2001) to persist in an "alpine style glaciers" landscape. Meanwhile, southern populations could also have survived at higher latitudes (Markgraf, 1993; Mathiasen and Premoli, 2010), even colonizing nearby islands (i.e. Isla de los Estados), due to the drop of sea level during the LGM (Rabassa et al., 2005). The high allelic richness, low level of inbreeding and a lack of evidence of genetic bottlenecks in populations around the contact zone add support to the meeting of colonization routes from these northern and southern refugia. Second, the contact zone might be the result of immigrants from local or nearby refugia. For example, Lago Guacho (43°S) and Nahuelpan (42°S) populations (pop *11-XI* and *8-VIII* respectively) harbor high levels of allelic richness and remained in unglaciated areas at central latitudes. In addition, the isolated population of San Martín (*12*, 43°S), which was also out of the ice and therefore a presumable relict, could have contributed to the admixture through population expansion.

Our results on nuclear and maternal markers (Soliani et al., 2012) are in agreement with previously reported evidence of contact zones for Patagonian taxa (e.g. in fishes, Zemlak et al., 2008; forest trees, Pastorino et al., 2009; herbs, Cosacov et al., 2010; Sérsic et al., 2011). On the other hand, a peculiar situation is observed for populations located at 51°13′ (*16*, *XVI*) and 51°31′S (*17*, *XVII*), which remained in ice-free areas but harbor low genetic diversity. These populations probably remained isolated for a long time originating a unique gene pool, similar to S. Martín (pop *12*) in Chubut province. Moreover, they could be particularly affected by the southern westerly wind belt, controlling and influencing climate patterns all over the Holocene (reviewed in Kilian and Lamy, 2012).

At the intra-specific level SGS showed different patterns for each species. *Nothofagus antarctica* is prompt to asexual reproduction and a low ability for long distance dispersal that might restrict gene flow and promote isolation by distance (Fig. S4). In agreement with cpDNA data (Soliani et al., 2012), *N. antarctica* showed higher levels of within population diversity than its congener, probably related to its resprouting capacity after disturbances (Premoli and Steinke, 2008; Veblen et al., 1996) and the longer generation time attributed to sprouters (Bond and Midgley, 2001). In contrast, *N. pumilio* is a strict outcrosser occupying the higher altitude niche, with no vegetative reproduction (Veblen et al., 1996) which can in turn favor the spread of the wind-dispersed pollen (long distance

dispersal). On the other hand, *N. pumilio* is more vulnerable to recruitment failure and problems associated with small population size, such as inbreeding (Bond and Midgley, 2001). The weak pattern of isolation by distance in *N. pumilio* fits with the broad distribution of some genetic clusters (both nSSR and cpDNA), and might also reflect the extensive past distribution of few genetic variants.

Estimation of pollen/seed mediated gene flow (Ennos, 1994) of *N. pumilio* and *N. antarctica*, based on the degree of standardize population differentiation from nuclear (F'_{ST}-SSRs) and maternal inherited markers (φ'_{ST}-cpDNA), showed a 10 times higher dispersal capacity of pollen versus seeds ($r = 13.3$ for *N. pumilio*; $r = 11.5$ for *N. antarctica*). The rates are comparable to the average estimated for a set of 93 species of Angiosperms from different geographical regions in the world ($r = 17$; Petit et al., 2005) but still substantially lower to the estimates reported in a recent study on *N. pumilio* (Mathiasen and Premoli, 2010).

4.2. Introgression and hybridization between species

In an attempt to describe population demography for *N. pumilio* and *N. antarctica* in a phylogeographic context, we provided inferences of past dynamics of hybridization and introgression. Due to extensive cpDNA haplotype sharing, the two species cannot be distinguished at the organelle level suggesting that their variants remained due to a low intra-species gene flow ($N_{ST\_N.pumilio} = 0.885$ and $N_{ST\_N\ antarctica} = 0.841$, Soliani et al., 2012) and a slow mutation rate. Conversely, newly developed nSSRs showed clear species separation (Fig. 2) and significant genetic differentiation (Table 1). Indeed, markers with high gene flow and high mutation rates within species facilitate species delimitation even when gene flow is extensive among species (Petit and Excoffier, 2009).

The between species admixture (*8–9* and *VIII–IX* populations) suggested the settlement of a hybrid zone in the boundary between the provinces of Rio Negro and Chubut (42°S) (Fig. 2). Interestingly, the highest inbreeding value ($F_{IS} = 0.157$) was detected in population *8* of *N. pumilio* (42°S), which could be due to Wahlund effect by an admixed substructure in the population. Indeed, natural hybridization between the two *Nothofagus* species studied here is well-known (e.g. Acosta and Premoli, 2010; Donoso Zeger, 2006), and is probably facilitated by overlapping niche space. At northern latitudes a clear ecological gradient determines the distribution of each species, *N. pumilio* being at higher altitudes than *N. antarctica*, thus conforming a west-east pattern of species dominance. The predominantly west-east directionality of winds could be the more relevant non biotic factor promoting hybrid zones. Toward the south, differences are less pronounced due to the decrease in elevation of the Andes Mountains, leading to an altitudinal coexistence of both species.

Flowering lag between different altitudes (Rusch, 1993) could be among the most important biotic aspect that foster hybridization in the between-species contact area. Accordingly, the high proportion of admixed individuals, mainly detected around 42–44°S, suggests a tight correspondence linking frequency with site location for the occurrence of hybridization events.

A recent phylogenetic study of *Nothofagus*, based on molecular dating of phylogenies, suggested an ancient process of introgression traced back to the Tertiary (~30 Myrs BP) (Premoli et al., 2012) by calling repeated events of chloroplast capture among all extant *Nothofagus* subgenera species (Acosta and Premoli, 2010). The assumed spatially extended and long lasting hybridization–introgression, that predates species divergence itself, should be even diluting species boundaries. Instead, authors conduct their arguments to the selection against hybrids in mixed-stands (Premoli et al., 2012) to counteract the effects of introgression affecting species delimitation.

At present time, species identity is maintained since an obviously differential ecological niche still occurs. Our genetic evidences are in agreement with the particular ecological differences of each studied taxa, reinforcing inter-species barriers, besides putting forward a more recent inter-specific contact, e.g. exclusive *cp*DNA haplotypes (Soliani et al., 2012), common SSRs alleles but highly contrasting frequencies (data not shown).

We do not discourage previously described theories but our results lead to contemporary (i.e. recent past) population demographic history, in relation to the LGM, to explain hybridization phenomena in the complex *N. pumilio–N. antarctica*. This is supported by a simple and parsimonious interpretation: a concordant geographical pattern of haplotype distribution, not only in the more frequent (probably ancestral) but also the less abundant variants (probably derived from the ancestral), in both species (Soliani et al., 2012). If the similarities were only the consequence of ancestral polymorphism, those patterns should show independence.

Furthermore, two different ABC analyses inferred similar timescales to explain hybridization in both species and therefore a common demographic history related to the LGM appears very likely. Although split and admixture times could have been more recent or ancient than the timescale based on median values (CI95%), inferred events still occurred around the Quaternary.

The admixed populations of *N. pumilio* (Pop*8* and *9*) were settled around 18,950 yrs BP. Similarly, hybrid populations diverged from an ancestral 'pure' population of *N. antarctica* within the same time (ABC2), about 26,700 years ago. According to the palaeoecological data of the region, the inferred median values are reasonable to explain the demographic history of *Nothofagus*.

Additionally, we also provide insights into the directionality of the historical hybridization process. In F_1 hybrids, *N. pumilio* could have acted as the pollen donor (Acosta and Premoli, 2010), with *N. antarctica* being the mother tree as suggested for *N. antarctica–N. dombeyii* hybrids (Stecconi et al., 2004). On the other hand, the similar number of individuals with a co-ancestry coefficient higher than 0.5 (q, STRUCTURE) suggests the occurrence of bidirectional backcrosses. Moreover, an equal contribution of both species to the hybrid genotypes of *N. pumilio* was estimated in ABC1 (*ra* from *N. antarctica* and *N. pumilio* to hybrid populations were 0.436 and 0.546, respectively).

We finally conceived another interpretation of the admixture-like pattern observed in several *N. antarctica* populations: a scenario of hybridization followed by repeated and directional backcrossing. As a pioneer and sprouting species (Premoli and Steinke, 2008), *N. antarctica* could have occupied larger areas during early colonization (Lepais et al., 2009). Therefore, backcrosses toward *N. antarctica* might be favored by the abundance of co-specific individuals and would also result in a high level of inbreeding (e.g. population *IX* of *N. antarctica*).

4.3. Conclusions

The recent settlement of the contact zone around 42°S in Patagonia, where different genetic lineages co-exist, could have been the origin of the most diverse populations of southern beeches (i.e. *Nothofagus*). This zone was affected by a glacial transition and holds a particular topography. Moreover, it includes hotspots of genetic diversity or admixed gene pools described for other species (Sérsic et al., 2011; Turchetto-Zolet et al., 2013), emphasizing the need of conservation polices (Petit et al., 2003). Additionally, we provide new insights into the past demographic history of species giving timescales for the occurrence of hybridization. Our results reinforce the idea of Patagonia being an important bio-geographical region.

Data accessibility

Individual multi-locus genotypes have been deposited in Dryad repository. Data package citation: Soliani C, Tsuda Y, Bagnoli F, Gallo LA, Vendramin GG, Marchelli P (2015) Data from: Halfway encounters: meeting points of colonization routes among the southern beeches Nothofagus pumilio and N. antarctica. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.r5303.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2015.01.006.

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