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Stand development stages and recruitment patterns influence fine-scale spatial genetic structure in two Patagonian *Nothofagus* species

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Abstract

Key message: *Nothofagus alpina* (Poepp. et Endl.) Oerst. and *Nothofagus obliqua* (Mirb.) Oerst forests have strong fine-scale spatial genetic structures. The intensity of genetic structure patterns differed according to species, stand development stages, life stages, and spatial arrangement of regeneration groups. This data becomes useful for forest management as it provides an understanding of how populations evolve as well as of the consequences of disturbances and enables the establishment of sampling strategies.

Context: The understanding of fine-scale spatial genetic structure in natural populations is useful for forest management. Although *Nothofagus alpina* (Poepp. et Endl.) Oerst. and *N. obliqua* (Mirb.) are important species of the Patagonian forest, little is known about the genetic structure of their populations.

Aims: The main objectives were to investigate the differences in fine-scale spatial genetic structure among mature tree populations of both species considering two stands at different development stages. Genetic structure was also evaluated among life stages and spatial distribution groups of regeneration within the old-growth stand.

Methods: Genetic structure was examined by microsatellite DNA analysis of regeneration and mature tree populations of both species (around 1300 individuals). Gene dispersal distance was additionally estimated.

Results: In both stands and species, strong fine-scale spatial genetic structure and short dispersal distance were found. This pattern was stronger in the early successional forest, in *N. obliqua* populations, in earlier life stages, and in scattered regeneration.

Conclusion: Stand development stages and recruitment patterns influence the fine-scale spatial genetic structure of both *Nothofagus* species. However, the genetic structure also differs between species.

Keywords: Fine-scale spatial genetic structure, Population density, Life stages, Natural thinning, Dispersal, Disturbance

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1 Introduction

The spatial genetic structure of plant populations has several implications for ecological and evolutionary genetics as well as for conservation biology (e.g., Larroque et al. 2019; Scribner et al. 2005; Silva de Olivera et al. 2020; Ueno et al. 2002). Understanding of the fine-scale spatial genetic structure (FSGS) in natural populations also provides a reference to detect the consequences of disturbances and to establish sampling strategies for ex situ and in situ conservation procedures (Doligez and Joly 1997; Buzatti et al. 2012; Sebbenn et al. 2008; Tarazi et al. 2010). Moreover, the genetic structure within a population follows spatial and temporal dynamics whose comprehension is one of the bases for understanding how populations evolve (Oddou-Muratorio et al. 2011; Piotti et al. 2012).

Sub-Antarctic forests are the southernmost temperate forests in the world and are dominated by *Nothofagus* species. The mixed forest of *Nothofagus alpina* [= *N. nervosa* (Poepp.et Endl.)] Oerst. (raulí) and *Nothofagus obliqua* (Mirb.) Oerst. (roble pellín) (Nothofagaceae) stands out for its ecological and economical importance (Donoso 1993). In Argentina, even though its natural distribution is located within the Lanin National Park, these mixed forests are managed due to their high-quality timber. Therefore, natural and anthropogenic disturbances varying in frequency and severity affect Patagonian *Nothofagus* forests shaping their dynamics (Veblen et al. 1996). After a large-scale disturbance (e.g., fire, blowdown), pure and mixed *Nothofagus* forests typically pass through four recognizable stand development stages (sensu Oliver and Larson 1996): (1) stand initiation, (2) stem exclusion, (3) understory reinitiation, and (4) old growth. In general, the stand initiation stage of succession follows a "catastrophic regeneration mode" characterized by large patches of even-aged trees derived from a few survivors from within or surrounding areas (Oliver and Larson 1996). This regeneration pattern is related to the heliophilous characteristic of these *Nothofagus* species (Loguercio et al. 2018; Veblen et al. 1996). During the stem exclusion stage, self-thinning drastically reduces population density but increases the biomass of remaining trees (Loguercio et al. 2018). In the understory reinitiation stage, understory replenishment may start with seedlings in a "gap phase regeneration mode," when gaps are opened up by the fall of mature individuals (Veblen et al. 1996). In the event that the stand is not affected by another large-scale disturbance, it will continue to the old-growth stage, in which canopy gaps are more frequent and produce environmental heterogeneity, promoting the establishment of regeneration and a forest with a mosaic of different ages (Veblen et al. 1996). During this succession, differences could be expected for FSGS as the population density is a key factor

affecting FSGS (e.g., Duminil et al. 2016; Hardy 2009; Sagnard et al. 2011). Accordingly, demographic processes, such as natural thinning, could affect fine-scale spatial genetic structure among different life stages (e.g., saplings, juveniles, mature trees) and across generations (Kitamura et al. 1997; Lian et al. 2008).

N. alpina and *N. obliqua* are both wind-pollinated, outcrossing, and anemochorous tree species that exhibit a mast seeding strategy every 2 to 4 years. However, regeneration recruitment in mixed forests also depends on niche differentiation between both species (Dezzotti et al. 2003; Sola et al. 2020a) which in turn influence spatial demographic structure. Therefore, if spatial heterogeneity in the demographic structure and in the recruitment pattern of the regeneration occurs, it may affect FGSS within populations. Moreover, the non-random spatial distribution of genotypes is influenced by pollen and seed dispersal distances, and stronger FSGS has been detected for species with lower dispersal distances (Asuka et al. 2005; Kitamura et al. 2018).

The significant fine-scale spatial genetic structure has been reported for populations of trees of *N. alpina* and *N. obliqua* (Marchelli et al. 2012; Sola et al. 2020b). This pattern was stronger for *N. obliqua* than for *N. alpina*, a result consistent with their differences in gene dispersal parameters (Sola et al. 2020b). For *N. pumilio*, FSGS was also detected in populations of trees at contrasting elevations, indicating that life history traits, including regeneration mode under distinct environmental (i.e., elevations) and historical (i.e., disturbances) factors, drive the spatial arrangement of genotypes in this species (Mathiasen and Premoli 2013). Furthermore, considering the mean nearest neighbor distance (MNND) as a threshold for distinguishing the effects of disturbances and biotic interactions, Fajardo et al. (2016) has revealed that at distances smaller than the MNND, trees of second-growth interior forests showed significantly lower relatedness probably due to competition, whereas trees of second-growth edge forests showed a positive and highly significant relatedness resulting from facilitation. However, despite their implications for understanding evolution and conservation biology of the species, comprehension of FSGS either in time (between stand development stages, generations, or life stages) or space (between spatial arrangements of individuals) is still scarce for mixed *Nothofagus* forests. Therefore, a special effort should be put into properly characterizing FSGS, considering forests with different stand development stages, life stages, and spatial distribution groups of regeneration in order to contribute to a deeper comprehension of their dynamics.

In this study, we have analyzed the patterns of fine-scale spatial genetic structure within mixed *Nothofagus* forests through genotyping of regeneration and mature

trees of *N. alpina* and *N. obliqua* with 12 and 11 micro-satellite loci, respectively. The study was performed in two stands at different developmental stages (stem exclusion and old growth). The main objectives were to analyze the FSGS (i) of mature tree populations of *N. alpina* and *N. obliqua* between both stands, (ii) among mature tree populations of each species within each stand, and (iii) among life stages and spatial distribution groups of regeneration of each species within the old-growth stand. Gene dispersal distance was additionally estimated based on FSGS data and compared between both stands. We hypothesize that there would be differences in the degree of fine-scale spatial genetic structure and gene dispersal distance between stands and species. We expect (i) stronger intensity of FSGS and shorter dispersal distance in the stem exclusion stand than in the old-growth stand due to its higher density of mature trees, (ii) a stronger FSGS for *N. obliqua* than for *N. alpina* due to its lower gene dispersal distance, (iii) discrepancies between life stages with a decreasing genetic structure from total regeneration to mature trees and from saplings to juveniles in the old-growth stand, and (iv) differences in the genetic structure between regeneration groups. The regeneration aggregated in patches would be more genetically similar and consequently more structured than the scattered regeneration.
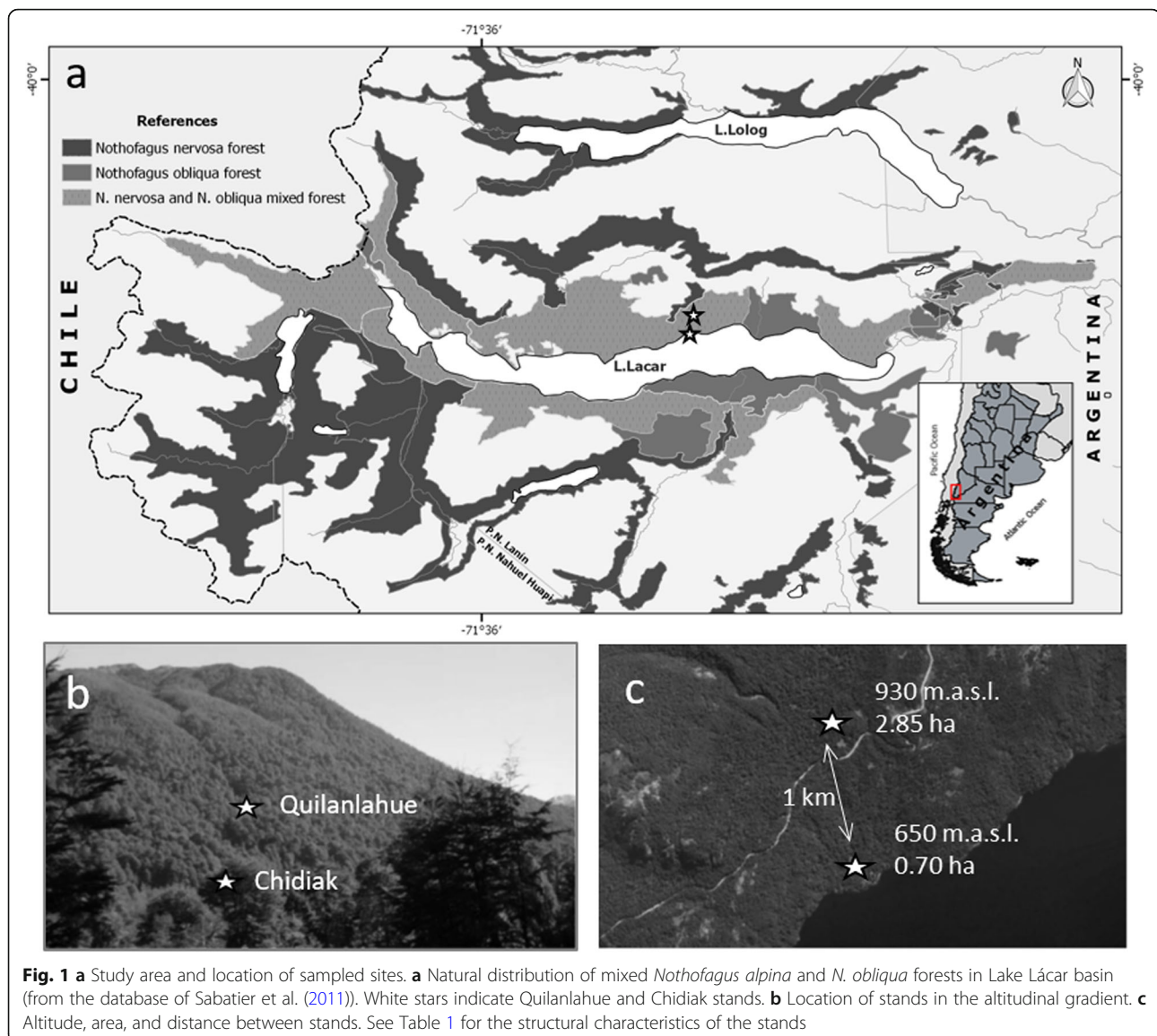
2 Material and methods

2.1 Study area and sampling design

In Argentina, mixed subantarctic *Nothofagus alpina* and *N. obliqua* forests occur along the eastern foothills of the Andes between 39° 29′–40° 22′ S and 71° 15′–71° 40′ W, in areas with annual precipitation of 1000–2000 mm and deep and drained volcanic soils (Loguercio et al. 2018). In this region, mixed *Nothofagus* forests are mainly in the understory reinitiation and old-growth stages, although there are also fewer forests in the stand initiation and stem exclusion stages (Loguercio et al. 2018). Two *Nothofagus* mixed stands (Quilanlahue and Chidiak local areas), from a network of Permanent Plots for Genetics, Ecological and Management Studies installed in 2009 in Lanín National Reserve (Neuquén province, Argentina), were selected (Fig. 1). These stands have differences in tree size and density of mature trees, as well as in regeneration patterns according to field observation. These differences were confirmed during this work (see Section 2.2). The stands are located in continuous forests in the middle of their distribution area, only 1 km apart from each other (Fig. 1). However, Quilanlahue is an old-growth stand, while Chidiak is a post-fire stand in the stem exclusion development stage, according to fire scares found on older trees (Marcelo Peñalba pers. com.). Silvicultural management was carried out in Quilanlahue in 1993, to reach a canopy cover

of around 40%. However, even before the silvicultural management, this stand showed lower density and higher size of mature trees than the Chidiak stand (Sola et al. 2015). Moreover, logging has not significantly influenced the genetic diversity and patterns of gene flow (Sola et al. 2016; Sola et al. 2020b). This result could be a consequence of the type of intervention, which was performed maintaining the original relative species composition and the homogenous spatial distribution of mature trees (Sola et al. 2020b). Therefore, we could compare both stands considering their contrasting forest structures.

2.2 Stand structure characterization

The spatial coordinates and dendrometric information (number of stems per hectare, diameter at breast height and tree heights, and ages) of mature trees from both species were previously determined for the Quilanlahue stand (Sola et al. 2015). The same methodology was used to recover spatial and dendrometric information in the Chidiak stand for this study. This information was used to make maps of individual locations and frequency distribution of mature tree ages. Total regeneration at Quilanlahue stand (individuals with a root collar diameter < 10 cm and height < 7 m) was sampled along 14 transects (5 m width, 100 m length) systematically distributed every 20 m. Regeneration sampled along each transect corresponded to two spatial patterns of regeneration recruitment (i.e., patches and scattered regeneration, Fig. 2) previously reported at this stand (Sola et al. 2016). For regeneration patches, a minimum of 30 individuals were sampled. The spatial coordinates of the regeneration corresponded to a systematic sampling grid every 10 m along transects, i.e., the regeneration near each grid point share the same x and y coordinates. Previously adjusted height/age linear functions (Sola et al. 2015) were used for age estimations for each species. Based on these estimations, two life stages, namely saplings (< 6 years and < 1.5 m of height) and juveniles (> 9 years and > 3.5 m of height), were identified with an intermediate group (6–9 years and 1.5–3.5 m of height) not clearly defined. For that reason, for FSGS analysis between life stages, regeneration of this intermediate group was excluded to avoid overlapping cohorts. Therefore, for the regeneration, FSGS analysis was performed considering (i) total regeneration (i.e., including all individuals sampled), (ii) two groups of spatial distribution (i.e., patches vs. scattered regeneration), and (iii) two different life stages (i.e., saplings vs. juveniles). Student's *t*-test was employed to evaluate the differences between the ages of the different regeneration groups, using Statgraphics v. 15.02.06. The Chidiak stand has no established regeneration; therefore, comparisons between life stages were only performed at the Quilanlahue stand.

Fig. 1 a Study area and location of sampled sites. **a** Natural distribution of mixed *Nothofagus alpina* and *N. obliqua* forests in Lake Lácar basin (from the database of Sabatier et al. (2011)). White stars indicate Quilanlahue and Chidiak stands. **b** Location of stands in the altitudinal gradient. **c** Altitude, area, and distance between stands. See Table 1 for the structural characteristics of the stands

2.3 Genetic characterization

Total DNA was extracted from leaf or bud tissue from 109 and 84 mature trees of *N. alpina* and *N. obliqua* from Quilanlahue and 74 and 85 from Chidiak, respectively. In the regeneration of Quilanlahue, 158 and 813 individuals of *N. alpina* and *N. obliqua* were sampled, respectively. We used 12 (*N. alpina*) and 11 (*N. obliqua*) polymorphic nuclear microsatellite (SSR) loci from the 15 SSRs previously genotyped (monomorphic loci were excluded) on both species (Sola et al. 2020b). The selected loci were 19SN, 13SN, 8SN, 32SN, 23SN, IN0230a (Torales et al. 2012), Notho214, Notho216 (El Mujtar et al. 2014), NnBio37, NnBio111 (Marchelli et al. 2008), NnBio11 (Azpilicueta et al. 2004), and Oakpum64 (Soliani et al. 2010). Considering that interspecific hybridization can influence dispersal estimations

(Oddou-Muratorio et al. 2001), we excluded individuals with hybrid genotype from adult and regeneration trees (El Mujtar et al. 2017). Inbreeding coefficient, null allele frequency, and genotyping error rates of microsatellite loci have been previously estimated by Sola et al. (2020b), confirming their quality for genetic studies.

To characterize the genetic diversity of both stands, we calculated the effective number of alleles (Ne, Brown and Weir 1983), allelic richness (Ar, El Mousadik and Petit 1996), and observed and expected heterozygosity (Ho and He respectively, Nei 1973) for each species using Fstat v. 2.9.3 (Goudet 2001) and Genalex (Peakall and Smouse 2006). A paired *t*-test was performed to evaluate the statistical significance of the differences between the genetic diversity estimates of each species between stands, using Statgraphics v. 15.02.06. Log
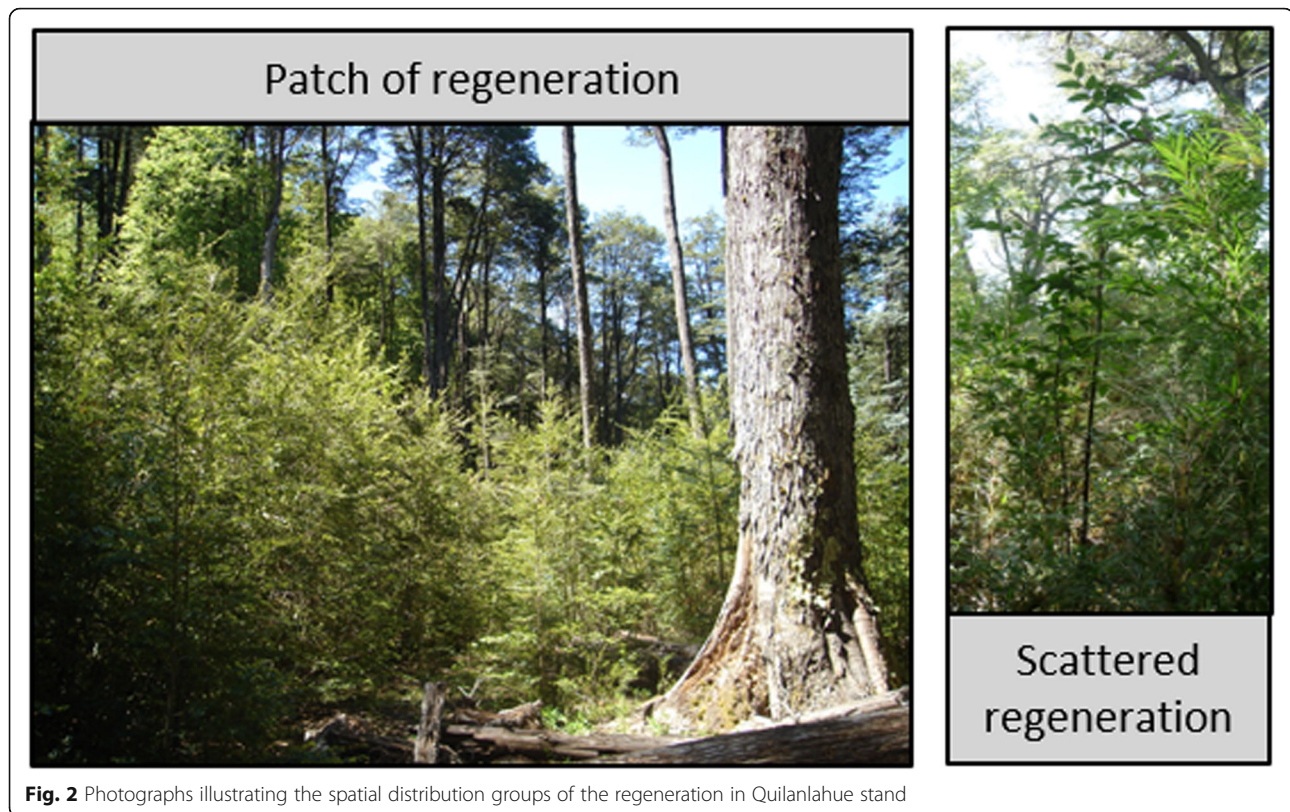
Fig. 2 Photographs illustrating the spatial distribution groups of the regeneration in Quilanlahue stand

transformations were used in the cases that normality was not achieved.

2.4 Fine-scale spatial genetic structure analysis

Fine-scale spatial genetic structure was assessed in both stands considering all mature trees and the regeneration following the procedure described in Vekemans and Hardy (2004) and previously applied in Sola et al. (2020b). Pairwise kinship coefficients (F_{ij}) were estimated between individuals using Nason's estimator of kinship (Loiselle et al. 1995), chosen due to its statistically robust properties (Vekemans and Hardy 2004), using the software SPAGEDI 1.4c (Hardy and Vekemans 2002). Estimations were performed considering five distance classes for which maximal distances are defined in such a way that the number of pairwise comparisons within each distance class is approximately constant (Hardy and Vekemans 2002). Furthermore, to illustrate the FSGS graphically, F_{ij} values were also averaged over the set of five distance classes. The coordinates of the regeneration were not individual; they corresponded to a systematic sampling grid every 10 m along transects, i.e., the regeneration near each grid point share the same x and y coordinates and was considered a spatial group for SPAGEDI. The program added a distance class for the pairwise coefficients between members of the

same group, the intra-group pairwise kinship coefficient (F_0). To test the overall difference of each species between stands, we performed a t-test for paired comparisons of the kinship coefficients and the Sp statistic among loci. Log transformations were used in the cases that normality was not achieved. Between species, only tendencies could be mentioned because different loci were used constraining paired tests.

Considering mature trees and assuming drift-dispersal equilibrium, we also estimated the gene dispersal distance, σ_g (σ^2_g is half the mean-squared parent-offspring distance), and the neighborhood size, $Nb = 4\pi d_e \, \sigma^2_g$ (d_e is an effective population density of reproductive individuals) following Vekemans and Hardy (2004) and previously applied in Sola et al. (2020b). The effective population density used was d_e = observed density (d_{obs}) for Chidiak, while it was d_e = 2 * d_{obs} for Quilanlahue, to take into consideration that d_{obs} was determined after forest management.

3 Results

3.1 Stand characterization

Quilanlahue showed a demographic structure characterized by older trees (t-test $p < 0.05$) and lower density than the Chidiak plot (Table 1, Fig. 3). Age classes for Quilanlahue followed an approximately normal distribution for both species, but with the highest frequencies in different age classes, showing

Table 1 Characterization of plots established at two stands of mixed *Nothofagus* forest

	Mean age[a] (years)		DBH (cm)		Density (Ind/ha)	
	N. alpina	*N. obliqua*	*N. alpina*	*N. obliqua*	*N. alpina*	*N. obliqua*
Quilanlahue	135 ± 67	205 ± 65	53 ± 30	74 ± 22	40	32
Chidiak	64 ± 32	62 ± 25	26 ± 16	27 ± 14	151	197

[a]Estimated considering the correlation model based on wood ring counts established by Sola et al. (2015)

N. alpina younger individuals than those of *N. obliqua* (*t*-test $p < 0.05$, Table 1, Fig. 3). In contrast, the age distribution in Chidiak was more skewed towards smaller ages, with no differences between species in the age of the highest frequencies (Table 1, Fig. 3). Stand structures detected for Quilanlahue and Chidiak plots were characteristics of old-growth and stem exclusion stand development stages, respectively. In Quilanlahue, 113 and 90 mature trees of *N. alpina*

and *N. obliqua* were mapped, and in Chidiak, 106 and 138, respectively (Fig. 4). The percentage of non-genotyped individuals was higher in Chidiak (36%) than in Quilanlahue (6%) (Fig. 4) due to the difficulties to reach treetops in the dense canopy.

In Quilanlahue, the age of total regeneration ranged from 3 to 17 years old, with saplings and juveniles (mean age of 5.15 and 11.15 years, respectively) representing 23% and 19% of the total regeneration,
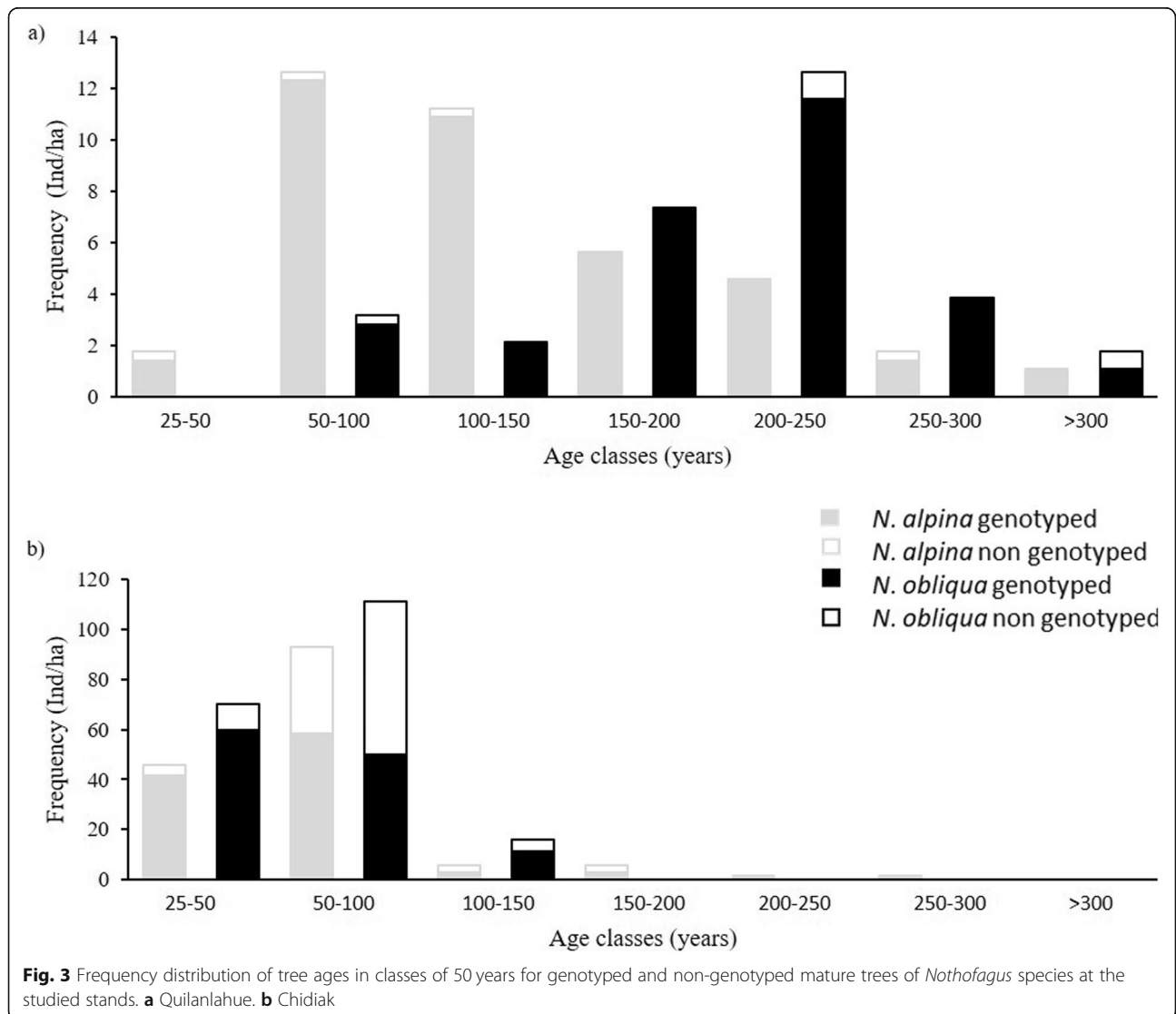

Fig. 3 Frequency distribution of tree ages in classes of 50 years for genotyped and non-genotyped mature trees of *Nothofagus* species at the studied stands. **a** Quilanlahue. **b** Chidiak
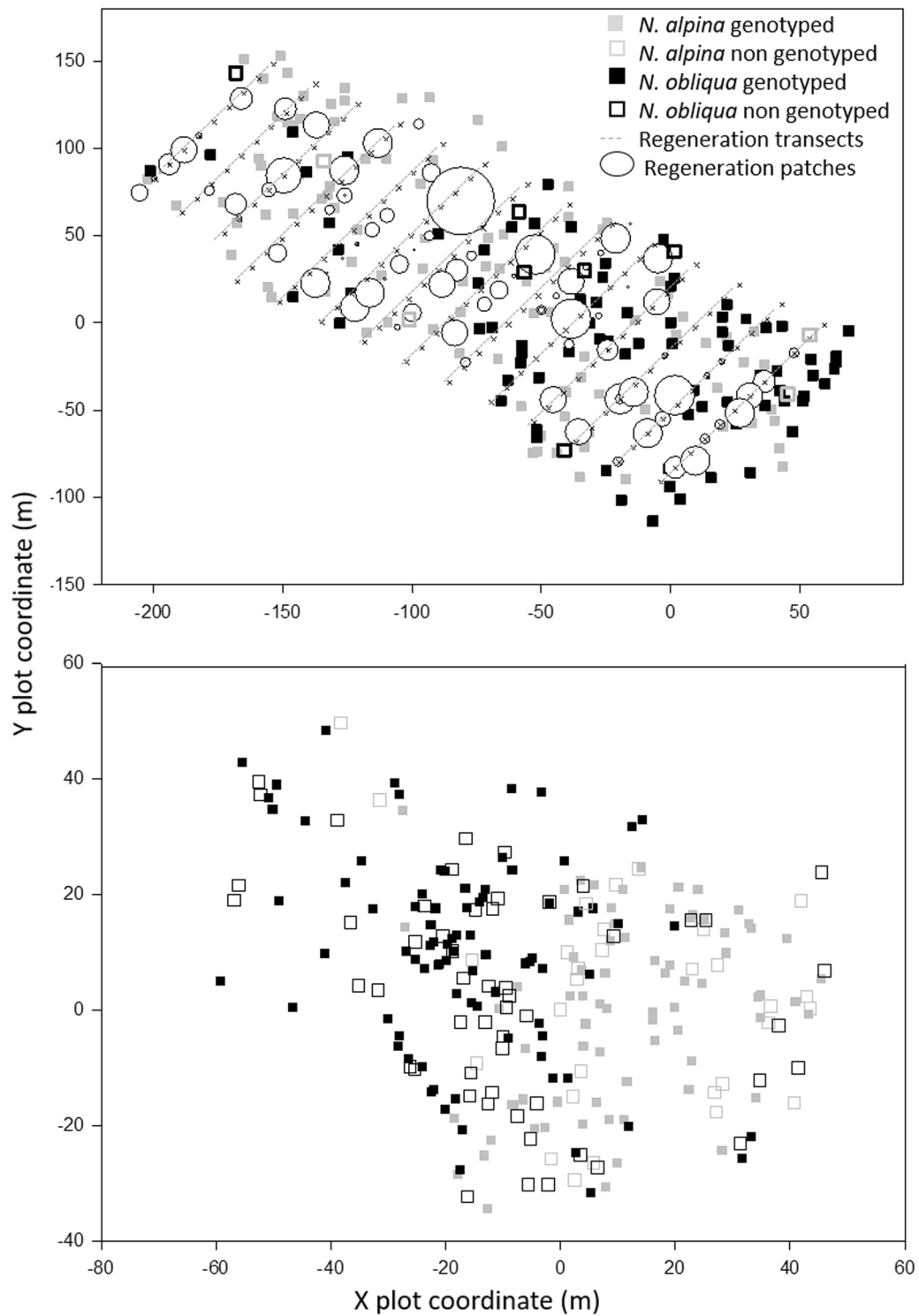
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Fig. 4 (See legend on next page.)

respectively (Fig. 5). Regeneration in patches and scattered represented 71% and 29% of the total regeneration, respectively (Table 2). As expected, considering the definition of the life stage categories (see Section 2), the statistical differences (*t*-test $p < 0.05$) between the age of saplings and juveniles were detected for both species (Table 2). The statistical differences (*t*-test $p < 0.05$) were also detected between the age of the scattered regeneration and the regeneration in patches, but with different patterns in each species (Table 2). The scattered regeneration of *N. alpina* was older than the regeneration in patches, while the inverse pattern was detected for *N. obliqua* (Table 2).

No significant differences in genetic diversity parameters were found between mature trees of *N. obliqua* between both stands (Table 3). In *N. alpina*, only the allelic richness of mature trees was higher in Quilanlahue than in Chidiak (paired *t*-test $p < 0.05$).

3.2 FSGS and gene flow between stands of *N. alpina* and *N. obliqua* at different development stages

FSGS was significant in both stands for both species but only at short distance, extended up to 50 m in Quilanlahue and up to 30 m in Chidiak (Table 4, Fig. 6). A tendency towards a higher *Sp* statistic was found in both species for Chidiak than for Quilanlahue (Table 4). However, the difference in *Sp* between stands was only statistically significant for *N. alpina* ($p < 0.05$). Gene dispersal distance and the neighborhood size were consequently shorter for Chidiak than for Quilanlahue. At each stand, a tendency of a stronger intensity of FSGS and shorter gene dispersal distance for *N. obliqua* than for *N. alpina* was detected (Table 4).

3.3 FSGS among life stages and spatial distribution groups of regeneration

Within Quilanlahue, a significant FSGS which extended up to 50 m was found in total regeneration of *N. alpina*
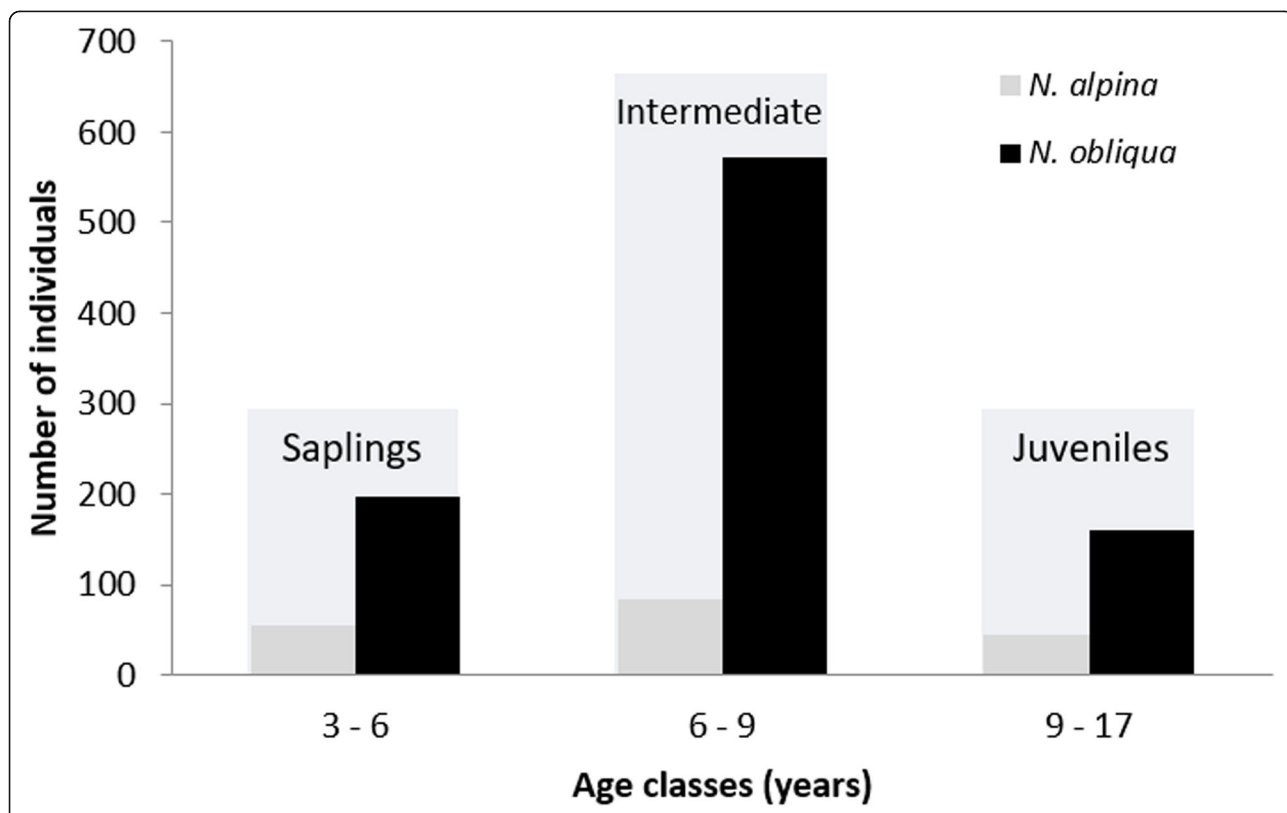

Fig. 5 Frequency distribution of regeneration ages of *Nothofagus* species in 3 predefined classes at the studied stand of Quilanlahue

Table 2 Mean age, SE, and number of individuals (*N*) of the regeneration analyzed in Quilanlahue considering three categories: total regeneration, life stages (i.e., saplings and juveniles), and spatial distribution groups (i.e., patches and scattered)

	Species (N)	Mean age (years)
Total regeneration	*N. alpina* (173)	7.69 ± 2.83
	N. obliqua (827)	7.43 ± 2.04
Saplings	*N. alpina* (51)	4.94 ± 0.65
	N. obliqua (176)	5.21 ± 0.58
Juveniles	*N. alpina* (43)	11.76 ± 2.08
	N. obliqua (146)	10.97 ± 1.63
Patches	*N. alpina* (89)	7.09 ± 2.18
	N. obliqua (623)	7.72 ± 2.13
Scattered	*N. alpina* (84)	8.32 ± 3.29
	N. obliqua (204)	6.70 ± 1.51

and *N. obliqua* (Table 5, Fig. 7). Between mature trees and total regeneration, there was no statistically significant difference of *Sp*, reflecting a similar strength of FSGS. However, the F_0 kinship coefficient in total regeneration was higher (*N. alpina*) or similar (*N. obliqua*) to the F_1 kinship coefficient of mature trees, showing a strong spatial genetic structure of total regeneration at very short distances in both species (≤ 10 m considering the systematic sampling grid we used).

Regarding the two cohorts of regeneration, in both species, a tendency of a higher F_0 kinship coefficient for saplings than for juveniles was detected (Table 5, Fig. 7). However, the difference was only significant for *N. obliqua* (paired *t*-test $p < 0.05$). With respect to the spatial distribution of the regeneration, we found that the scattered regeneration presented higher values of F_0 than the regeneration in patches in both species (paired *t*-test $p < 0.05$) (Table 5, Fig. 7). The comparison of *Sp* between both cohorts (saplings vs. juveniles) and spatial distribution groups (patches vs. scattered regeneration) could not be performed, as most of the F_1 kinship

Table 3 Genetic diversity of mature trees of *N. alpina* and *N. obliqua* for Chidiak and Quilanlahue

Species	Population	N	Ne	AR	Ho	He
N. alpina	Chidiak	76	2.036	4.374*	0.487	0.464
	Quilanlahue	109	2.284	4.703*	0.466	0.497
N. obliqua	Chidiak	85	2.072	3.562	0.273	0.394
	Quilanlahue	84	2.142	3.540	0.227	0.375

Diversity parameters averaged over all loci

N sample size (number of individuals), *Ne* number of effective alleles, *AR* allelic richness (*N* = 71 for *N. alpina* and *N* = 45 for *N. obliqua*), *Ho* observed heterozygosity, *HE* expected heterozygosity or Nei diversity (Nei 1973)

*Statistically significant differences = $p < 0.05$

coefficients were not significant indicating the absence of structure at distances higher than 10 m (Table 5, Fig. 7).

4 Discussion

To our knowledge, this is the first empirical study to analyze both the temporal and spatial change in fine-scale spatial genetic structure in *N. alpina* and *N. obliqua* mixed forests. Although the stand structures of the old-growth and the stem exclusion forests were very different, mature trees of both types of forest showed similar genetic diversity and a significant FSGS with higher relatedness at short distances, suggesting non-homogeneous (and restricted) gene flow. This result agrees with previous reports in these and in other *Nothofagus* species (Marchelli et al. 2012; Sola et al. 2020b; Fajardo et al. 2016; Mathiasen and Premoli 2013), suggesting that *Nothofagus* are characterized by short dispersal distance and strong fine-scale spatial genetic structure. However, this restricted pattern was stronger for mature tree populations of the early successional forest (Chidiak, stem exclusion stage) than for the old-growth stand (Quilanlahue) indicating a decrease in genetic structure. A tendency of higher genetic structure for *N. obliqua* than for *N. alpina* mature trees was also found in both stands, a result that agrees with the lower gene dispersal distance of *N. obliqua*. Within the old-growth stand, a decreasing FSGS across life stages was also detected (i.e., total regeneration to mature trees, saplings to juveniles). Additionally, in this stand, individuals with scattered establishment showed stronger spatial genetic structure than those established in patches. All these results increase our understanding of FSGS for *N. alpina* and *N. obliqua* mixed forests.

The data provided here would be helpful for further studies aiming to understand the disturbance impact on FSGS or to follow FSGS changes over time. Both sites are part of a network of permanent plots and have mature trees mapped and genotyped; therefore, reanalyzing data by excluding dead individuals over time will be useful to evaluate the temporal dynamics of FSGS in both species. Our results may also be used to guide management recommendations. For example, according to the strong FSGS determined for both species, a harvesting strategy based on coarse removal of the entire patches of trees is not recommended, because it may affect genetic diversity. Moreover, it would be useful to evaluate how management can impact FSGS. For example, the impact of management on FSGS could be evaluated in the stem exclusion stand, before management implementation, by reanalyzing data excluding selected trees (i.e., trees selected for cutting). Our results also have direct implications for conservation, restoration, and breeding programs. Information on FSGS levels is important for

Table 4 Estimates of FSGS and gene flow for mature tree populations of each species for five distance classes: average kinship coefficient between individuals of the first distance class (F_1), FSGS intensity (Sp) and its standard error in parentheses, neighborhood size (Nb), gene dispersal distance (σ_e), and 95% confidence interval for effective density (Quilanlahue $d_e = d_{obs} * 2$, Chidiak $d_e = d_{obs}$)

Species (number of pairs)		Quilanlahue		Chidiak	
		N. alpina (1177)	N. obliqua (697)	N. alpina (570)	N. obliqua (714)
FSGS parameters	F_1	0.0070* (0.0027)	0.0203* (0.0105)	0.0125* (0.006)	0.0184* (0.0085)
	Sp	0.0089 (0.0028)	0.0143 (0.0057)	0.0109 (0.0045)	0.0161 (0.0063)
Gene dispersal parameters	Nb	261 (69–308)	65 (39–366)	72 (50–544)	33 (35–303)
	σ_g	51 (26–55)	28 (21–66)	13 (11–36)	9 (10–29)

*p-value of the 2-sided test was lower than 0.05. This test was performed to evaluate if the kinship coefficient was different from the permuted value

seed collections to develop reforestation strategies (Bittencourt and Sebbenn 2007; Bizoux et al. 2009; Buzatti et al. 2012). In comparison with panmictic populations, seed collection in populations exhibiting FSGS requires greater distances among trees and large sample sizes to avoid collecting seeds of related trees that represent only a subset of the genetic diversity. Therefore, in mixed *Nothofagus* forests, seeds must be collected from trees at least 50 m apart in order to reduce genetic similarity among seed trees and to obtain a representative sample. For ex situ gene conservation and/or reforestation programs where one of the goals is to maintain genetic variability and limit consanguinity, the abovementioned distance between trees should be maintained.

Patterns of fine-scale spatial genetic structure detected in these mixed forests could be established by the effect of several drivers, such as dispersal distance, population density, and natural thinning. Many studies have reported restricted gene flow as the major factor affecting FSGS (Chung et al. 2003; Kitamura et al. 2018; Troupin et al. 2006). In this sense, short pollen and seed dispersal distances have been reported in *N. obliqua* and in *N. alpina* (Sola et al. 2020b), a characteristic that could explain the pattern detected in both stands. The dispersal capacity of each species after the disturbance is likely to have determined the population age structure within the sites. In Chidiak, the narrower and exponential age distribution of mature trees (mean age of 63 years)
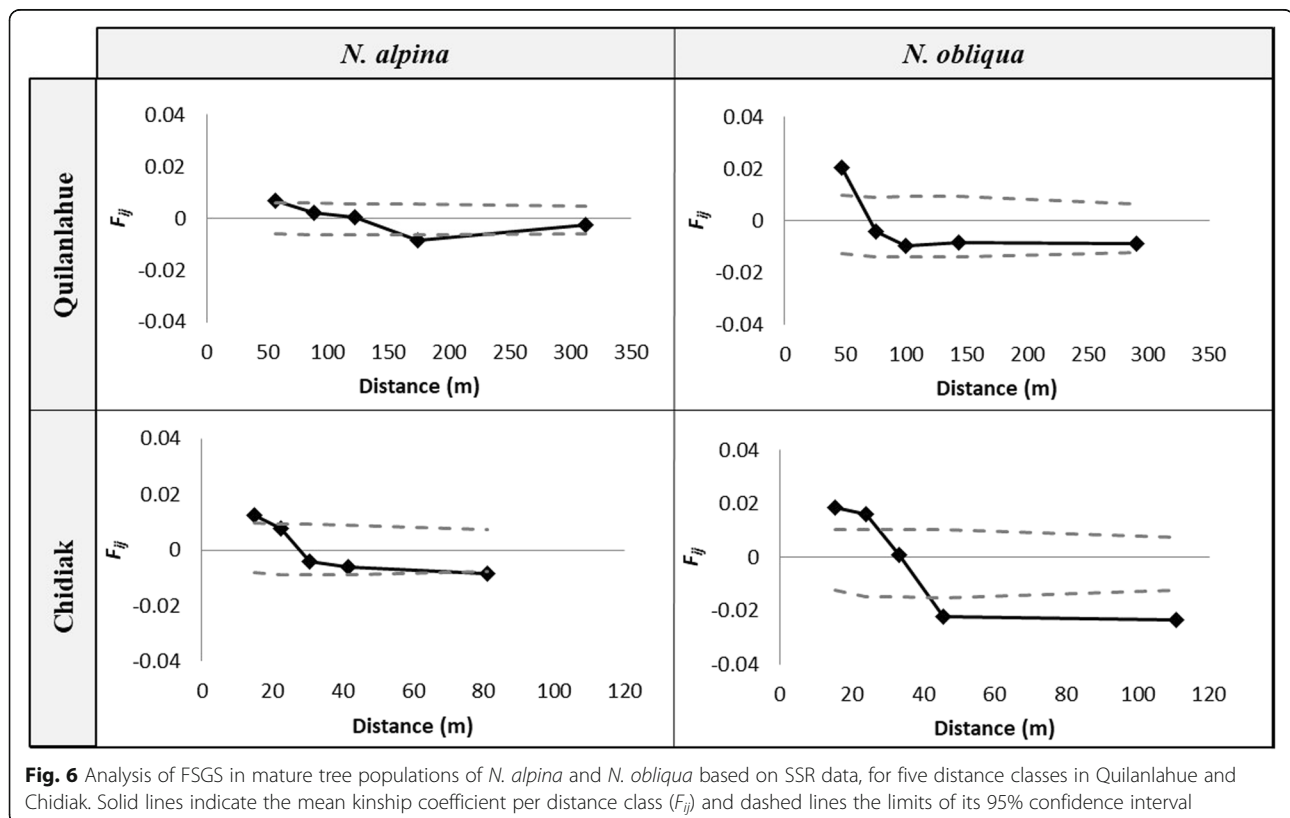

Fig. 6 Analysis of FSGS in mature tree populations of *N. alpina* and *N. obliqua* based on SSR data, for five distance classes in Quilanlahue and Chidiak. Solid lines indicate the mean kinship coefficient per distance class (F_{ij}) and dashed lines the limits of its 95% confidence interval

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Table 5 Estimates of fine-scale spatial genetic structure (FSGS) for the regeneration of Quilanlahue: average kinship coefficient between individuals of the first distance class (F_1) and FSGS intensity (Sp) and its standard error in parentheses for 10 distance classes. The average intra-group kinship coefficient (F_0) is also informed

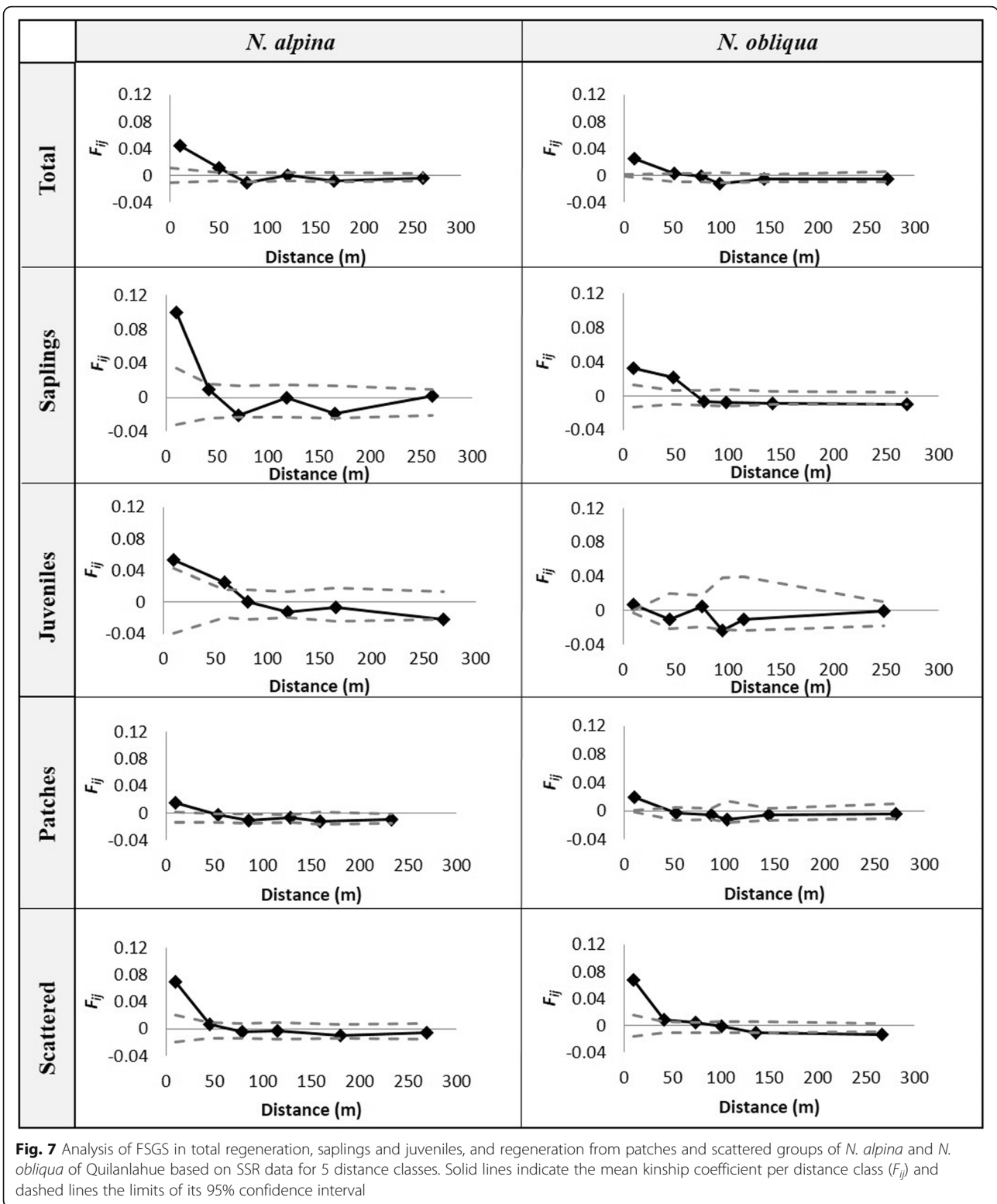| Species	N. alpina					N. obliqua				
Regeneration (number of pairs)	Total regeneration (2387)	Saplings (254)	Juveniles (174)	Patches (717)	Scattered (670)	Total regeneration (57,701)	Saplings (2958)	Juveniles (1100)	Patches (30,529)	Scattered (4051)
F_1	0.0110* (0.0060)	0.0096 (0.0115)	0.0244* (0.0107)	0.0032 (0.0018)	0.0070 (0.0087)	0.0035* (0.0032)	0.0212* (0.0060)	− 0.0103 (0.0063)	− 0.0033 (0.0038)	0.0086* (0.0034)
Sp	0.0065 (0.0047)	0.0057 (0.0093)	0.0266 (0.0127)	0.0072 (0.0013)	0.0072 (0.0066)	0.0082 (0.0037)	0.0180 (0.0058)	0.0027 (0.0020)	0.0139 (0.0037)	0.0140 (0.0064)
F_0	0.0442*	0.1000*	0.0528*	0.0151*	0.0695*	0.0252*	0.0328*	0.0071*	0.0197*	0.0679*

*p-value of the 2-sided test was lower than 0.05. This test was performed to evaluate if the kinship coefficient was different from the permuted value

indicates a more recent establishment, derived after a fire disturbance probably by an influx of seeds from survivors from within or surrounding areas. Self-thinning and gap formation seem to be recently starting in this stand, according to the high density of trees (348 trees/ha) and the moderate frequency of intermediates and suppressed trees (40% of mature trees, unpublished data). Therefore, the impact of these factors is yet scarce, possibly explaining the delayed dilution of the post-disturbance spatial genetic structure. Populations with even age structure or non-overlapping generations are expected to have more spatial structure than populations with multiple overlapping generations (Doligez et al. 1998; Jones and Hubbell 2006; Schnabel et al. 1991). Hence, in Quilanlahue, the wider and more flattened age distribution (mean age 170 years) indicates that mature trees were recruited over a longer period probably by high outcrossing rates and seed sources, leading to a weaker spatial genetic structure. Likewise, the lower density of this stand (72 trees/ha) indicates that this weaker pattern could also be the result of self-thinning. This pattern is suggested by the presence of gaps opened by the fall of senescent old-growth individuals and the low current diameter growth (around 0.2 cm per year, Sola et al. (2016)). The stronger spatial genetic structure in early successional stages than in older ones, most likely due to density-dependent processes that result in thinning, has also been previously confirmed in other species (Kitamura et al. 2018; Ueno et al. 2002).

Short dispersal distance and natural thinning could also be determining the decrease of FSGS across life stages within the old-growth stand. For other tree species, this pattern of decrease in structure and relatedness from juveniles to mature trees has been related to limited dispersal near the parent plant followed by natural thinning (Berens et al. 2014; Chung et al. 2003; Jones et al. 2005; Kitamura et al. 2018). In this sense, it is known that limited gene flow reinforces FSGS across generations (Moran and Clark 2012; Smouse et al. 2008). In the old-growth stand, saplings were more structured than juveniles indicating the possible effect of self-thinning on the weakening of FSGS of these species. However, validation by studies of regeneration dynamics, determining mortality, and using individual spatial coordinates would be required. This would allow a more accurate comparison of the strength of the FSGS in different life stages and the relation with demographic factors. Also, the processes of structuring, dispersal, and genetic diversity should be analyzed, in a greater number of stands subject to different disturbances and therefore in different development stages in order to contribute to our results with analyses on different conditions.

The community structure of regeneration in *Nothofagus* stands is heavily influenced by the abundance and size of canopy gaps (Dezzotti et al. 2003; Sola et al. 2020a) but also by eco-physiological interspecific differences (Loguercio et al. 2018). It is known that after an opening, the pioneer species *N. obliqua* is the first to establish (Sola et al. 2015; Sola et al. 2020a). This species tolerates full sunlight better than the mid-shade-tolerant species *N. alpina* (Varela et al. 2012), which establish later with a less aggregated pattern (Dezzotti 2008; Sola et al. 2020a). This occurs early during the regeneration recruitment, and it would explain the variability in stand structure (e.g., species composition, densities, and ages) within *Nothofagus* mixed forests (Donoso et al. 2013; Sola et al. 2016, 2020a; Weinberger and Ramírez 2001). The variation in the intensity of the spatial pattern over the study site, i.e., in some areas where regeneration density of each species was higher than in others (patches and scattered regeneration), indicates that there was micro-environmental heterogeneity generating the different spatial distribution of species in favorable establishment sites, which could account for spatial aggregation (Sola et al. 2015, 2020a). Although the influence of micro-environmental conditions on regeneration establishment has not been evaluated in this work, it was previously analyzed in the same area (Sola et al. 2020a). This study, based on a different sampling strategy, has revealed that *N. obliqua*

Fig. 7 Analysis of FSGS in total regeneration, saplings and juveniles, and regeneration from patches and scattered groups of *N. alpina* and *N. obliqua* of Quilanlahue based on SSR data for 5 distance classes. Solid lines indicate the mean kinship coefficient per distance class (F_{ij}) and dashed lines the limits of its 95% confidence interval

regeneration was associated with open microsites and *N. alpina* regeneration with shady conditions with moderate water stress. Therefore, micro-environmental heterogeneity with respect to

recruitment and establishment requirements may also be a key factor in the FSGS (Troupin et al. 2006). In this case, the scattered regeneration of *N. obliqua* was the fifth of the patchy regeneration, and it was also

the most structured, as only few individuals were able to establish under these more shaded conditions. On the other hand, *N. alpina*, the most tolerant to shade of the *Nothofagus* species, presented the seventh of the regeneration shown by *N. obliqua* in the opened patches. Hence, selection in response to the fine-scale environmental heterogeneity and/or variation in the level of adaptation of individuals to it could result in FSGS (Epperson 1992).

We have found that regeneration with scattered establishment showed higher spatial genetic structure than regeneration established in patches, rejecting our prediction. These patches had the highest regeneration density and were distributed throughout the entire site. Therefore, they would be formed by the contribution of a large part of the mature trees surrounding the gaps and then favoring less structuring. Ueno et al. (2002) previously reported in other species that overlapping seed shadows in canopy gaps may lead to weaker genetic structure. On the contrary, the scattered regeneration would be formed by the contribution of the few and closer neighboring mature trees of microsites with closer canopy, thus being more related. In continuous vegetated areas, wind-dispersed species may exhibit stronger FSGS than in open areas, since seed dispersal may be reduced due to less intense winds within continuous vegetation (Nathan et al. 2012) and disruption of seed autorotation by collision with trees (Guries and Nordheim 1984).

It should also be noted that the age of the scattered vs. the patched regeneration was different in each species. In *N. obliqua*, the oldest regeneration was found in patches, indicating that this species was the first to establish when gaps were formed, while in *N. alpina*, the oldest regeneration was that of the scattered individuals, indicating that this species was established first under the remaining canopy of trees. This temporality in the establishment was also previously detected by Sola et al. 2020a in the same area based on a different sampling. Therefore, in addition to spatial separation, regeneration in the old-growth stand was separated temporally. Such temporal separation may result in differences in genetic composition and structure between them, which in turn could affect the evolutionary potential of a species (Ueno et al. 2002). All these results suggest that the eco-physiological characteristics of these mixed forest species are one of the main causes of the fine-scale spatial genetic structure by virtue of the different dynamics of the species after distinct disturbances, expressed in densities and ages. The results also suggest that in species with short dispersal patterns, such structure in the progeny transcends the generations and results in a substantial buildup of isolation by distance over repeated generations of the life cycle.

Globally, our results suggest (i) that population density could be shaping FSGS patterns, (ii) that inter-species differences are maintained across stand development stages, (iii) that natural thinning could be shaping FSGS across life stages, and (iv) that spatial patterns of regeneration arrangement could be related to inter-species differences in eco-physiological requirements and, consequently, in temporal establishment. However, understanding the drivers of the detected patterns would require FSGS evaluation in new stands, covering different development stages and considering different life stages and regeneration arrangements. Comprehending the ways in which species biology and disturbances interact is critical in making appropriate decisions about forest conservation which will best contribute to long-term forest sustainability. This study provides supporting data for forest management.

5 Conclusion

This study has revealed fine-scale spatial genetic structure, short dispersal distance, and similar genetic diversity in two *N. alpina* and *N. obliqua* stands. Stronger FSGS for the early successional stand and a decrease in structure and relatedness from regeneration to mature trees for the old-growth stand have also been detected. These results provide valuable data to follow FSGS changes over time and for further studies aiming to understand the disturbance impact on FSGS. Furthermore, they would be useful to elucidate the effect of patterns of dispersal, natural thinning, population density, life stages, and spatial arrangement of individuals on the FSGS of other forests. These results also have important implications for seed collection strategies for ex situ conservation of *Nothofagus* tree breeding and reforestation. The presence of spatial genetic structure in the stands indicates that seeds for ex situ conservation or tree breeding should be collected from trees at least 50 m apart in order to reduce relatedness among harvested seed trees.

Authors' contributions
GS, VEM, and PM conceived the experiment. GS and VEM carried out the FSGS analyses. LG proposed and contributed to the establishment of the permanent plots used in this study. GS, VEM, and PM wrote the manuscript. LG and LC reviewed the manuscript. All authors approved the final manuscript.

Availability of data and materials
The complete datasets of Quilanlahue and Chidiak stands is available at http://hdl.handle.net/20.500.12123/11152

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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