Regeneration mode affects spatial genetic structure of Nothofagus dombeyi forests

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Abstract

Disturbance may generate population bottlenecks by reducing population size and the number of founders establishing a new colony. We tested the hypothesis that the scale of disturbance affects the levels of genetic diversity and the spatial distribution of genotypes in naturally regenerating stands of Nothofagus dombeyi, an evergreen angiosperm tree, in northwestern Patagonia. At similar spatial scales, we predicted that old-growth stands characterized by fine-scale gap phase dynamics would be genetically diverse due to restricted gene flow among temporal and spatially isolated gaps. In contrast, young massively regenerated postfire cohorts resulting from coarse-scale disturbances would be genetically more homogeneous. At each of three paired old-growth and postfire stands a minimum of 50 trees were mapped and sampled within 1 ha. Fresh tissue was collected for isozyme analysis from a total of 361 trees along with tree cores and diameters. Tree age distributions reflected the dominant modes of regeneration. Six out of nine analysed loci were polymorphic. Mean genetic diversity parameters were greater but not significant in mature stands. Fixation indices suggested significant heterozygous deficit at two-thirds of possible tests indicating a Wahlund effect due to local recruitment of related seeds. $F_{ST}$ indicated moderate between-stand divergence. Mature stands concentrated half of positively like joins and yielded significant ($P < 0.05$) autocorrelation coefficients at small distance classes (< 20 m). Fine-scale patch dynamics within mature stands favours the maintenance of fine-scale genetic structure as a result of shade intolerance and local seed dispersal. Conversely, postfire stands suffer the effects of genetic drift given that a few reproductive trees produce a somewhat impoverished and genetically uniform progeny. Bottleneck effects will depend upon the density of remnant trees which could also be a function of the severity of fire.

Keywords: disturbance, isozyme variation, Nothofagus, South America, spatial autocorrelation

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Introduction

There is now good empirical evidence that a large proportion of the genetic diversity of woody plant species resides within populations (Hamrick et al. 1992). Despite a growing number of studies that explore patterns of genetic structuring within populations, it is more difficult to make broad generalizations about how this large amount of genetic variation is substructured in space. Studies have yielded variable results from nearly random structures (Epperson & Allard 1989) to weak or moderate evidence of fine genetic structure within populations (e.g. Boyle et al. 1990; Parker et al. 2001; Chung et al. 2002). Clearly, the development of nonrandom spatial genetic structures within plant populations is regulated by complex combinations of microevolutionary processes. These include limited pollen and seed movement and variable microhabitat selection (Epperson 1993) closely linked with ecological and demographic processes that affect spatial and temporal patterns of adult mortality and seedling recruitment (Hamrick & Nason 1996).

Despite recognition that historical contingencies that affect stand dynamics such as disturbance events may have profound consequences on genetic substructuring of tree populations (Rice & Jain 1985), few studies have...
formally addressed this issue (Boyle et al. 1990; Knowles et al. 1992; Parker et al. 2001). Disturbance may pose important population bottlenecks by drastically reducing population size so that a small number of founders establish a colony (Vrijenhoek 1985). Reduction in genetic variability is counterbalanced if migration from surrounding undisturbed areas is possible. Clearly, the size and/or severity of disturbance may have a threefold effect on genetic structuring: (i) mortality regulates the population size of potential seed sources; (ii) disturbance affects the probability that new immigrants will colonize the site; and (iii) disturbance modifies local spatial heterogeneity of the environment which in turn modifies processes of microhabitat selection.

For tree populations, small canopy gaps caused by the death of one or a few dominant trees constitute relatively low severity disturbances of small spatial extent which leave abundant neighbouring survivors as seed sources. This often leads to a pattern of tree regeneration referred to as a fine-scale gap-phase mode of regeneration (Veblen 1992). Where fine-scale gap creation is frequent, such as over large surface areas, this mode of regeneration results in a temporally relatively continuous pattern of new establishment. In contrast, infrequent, coarse-scale disturbances that are of severe intensity over large surface areas often result in few survivors from within the disturbed area and may result in new colonization of the site by the same or other tree species in a pulse-like (even aged) mode over large areas. This is termed a catastrophic mode of regeneration and can be recognized by large patches of even-aged trees (Veblen 1992).

Perry & Knowles (1991) predicted that species with a dominant catastrophic mode of regeneration (e.g. fire-dependent, obligate seeding, nonserotinous species) would favour a state of genetic randomness whereas species with continuous regeneration mode within small gaps would promote fine-scale genetic structuring. This will be particularly the case in mature stands of species with local seed dispersal and a few adult trees contributing propagules to any given gap. Empirical evidence that supports this generalization comes from descriptive studies at the stand level of different species or comparative studies of populations with different stand histories. Epperson & Allard (1989) found a random distribution of genotypes in lodgepole pine stands and attribute this to long-distance pollen and seed dispersal. In contrast, Perry & Knowles (1991) found moderate degrees of genetic structuring in gap-phase replacing sugar maple (Acer saccharum) stands. Genetic differentiation among ages of trees or tree cohorts that have colonized coexisting gaps is proposed as one of the genetic structuring mechanisms. Knowles et al. (1992) found higher genetic structuring in a tamarack (Larix laricina) clearcut with ‘remnant’ seeds or seedlings than in L. laricina oldfield population where recolonization should have occurred from off-site sources. The authors attribute spatial genetic structuring in the former partially to genetic structuring of the previous remnant generation. Boyle et al. (1990) found a lowland ‘mature’ black spruce Picea mariana population to be more genetically clustered and less inbred than an upland postfire stand. It is suggested that in absence of large-scale disturbances clustering of genetically related seedlings close to parent trees would increase genetic structuring. Finally, the most formal approach addressing disturbance influences on genetic structuring is by Parker et al. (2001) who compared populations of two varieties of Pinus clausa with known differences in regeneration mode. Whereas weak genetic structure was found in young postfire cohorts due to limited seed dispersal, no apparent aggregation of similar genotypes occurred in older cohorts as a result of competitive thinning (Parker et al. 2001).

Unfortunately this evidence has to a large extent lacked adequate site replication or, when replication was present, variation in the development of fine-scale genetic structuring was evident (Parker et al. 2001). These shortcomings have prevented attribution of different genetic structures to specific causes such as disturbance history. On the other hand, other well site-replicated studies (Leonardi & Menozzi 1996) have lacked research designs to identify a priori mechanistic hypotheses of causal factors that may determine differences in genetic substructuring.

In northern Patagonia Nothofagus dombeyi is the dominant montane (< 1000 m a.s.l.) tree species. It exhibits geographical variation in regeneration modes (Veblen 1989a) along a steep west-to-east precipitation gradient (c. 3000 to < 1000 mm of annual rainfall). Coarse-scale regeneration follows disturbances of tectonic origin in the Vadivian rainforest (Veblen & Ashton 1978), fine scale gap-phase regeneration occurs in medium-sized treefall gaps in mesic monospecific Andean forests (Veblen 1985), and coarse-scale postfire regeneration occurs on the drier eastern slopes of the Andes in northwestern Patagonia (Veblen & Lorenz 1987; Veblen et al. 1992; Kitzberger 1994). At intermediate positions within this gradient in the Argentinean Andes both old-growth treefall gap-dominated and young large postfire cohorts coexist side by side. This natural setting offers the unique opportunity to examine the genetic consequences of these two contrasting regeneration modes.

In this study, we test the hypothesis that the scale of disturbance affects the levels of genetic diversity and the spatial distribution of genotypes in natural-regenerating stands of Nothofagus dombeyi in northwestern Patagonia. Analysed at similar spatial scales, we predict that coarse-scale disturbances will produce relatively depauperate and more genetically homogeneous stands due to genetic bottlenecks associated with a reduced number of remnant seed trees that survived the fire. Conversely, those stands under fine-scale gap-phase dynamics will be relatively diverse and genetically heterogeneous due to restricted gene flow among temporally and spatially isolated gaps. We also
expect that biparental inbreeding due to limited seed dispersal in *Nothofagus dombeyi* will reinforce genetic structure particularly if a small number of trees regenerate seedlings.

**Materials and methods**

**Study species**

*Nothofagus dombeyi* is an evergreen angiosperm that characterizes mesic forests of southern South America. It has a wide latitudinal range from Valdivian rainforests (39°S to 41°S), to southern temperate and Magellanic forests (41° to 44°S). *Nothofagus dombeyi* can attain 4 m in diameter and 40 m in height, and is the dominant species of the overstorey with a maximum longevity of c. 500–600 year (Veblen et al. 1996). *Nothofagus dombeyi* is the most shade-intolerant tree in our study area (Kitzberger 1994) and regeneration depends on gap formation. *Nothofagus dombeyi* massively regenerates after fire as discrete even-aged cohorts (Veblen & Lorenz 1994) even when only a few remnant trees are left. In mature (> 150 years old) *N. dombeyi* forests single and multiple treefalls provide potential regeneration opportunities. Within such gaps the understory bamboo *Chusquea culeou* attains high dominance and vigor producing thickets of up to 4 m in height. Despite the dominance of *C. culeou* and in absence of other more shade tolerant trees, *N. dombeyi* is able to regenerate within these gaps (Veblen 1989b). Seed production is highly erratic with years of low production and others with abundant seed rain (e.g. 880 and 17 000 seeds/m² measured along 1970 and 1971 in the Chilean Lake District, respectively; Burschel et al. 1976). As with many mast seedling trees, seed viability is positively related to seed production (Veblen et al. 1996).

*Nothofagus dombeyi* is wind pollinated and has a wide and relatively continuous range resulting in elevated polymorphism (P = 50%), and gene diversity (HT = 0.228) (Premoli 1997). Available estimates of outcrossing rates from three populations, although on a limited number of families, ranged between 0.87 and 1.04 suggesting that some selfing occurs in this species (Premoli 1996). *Nothofagus dombeyi* is monoecious and has limited seed movement, since its small achenes are mainly gravity dispersed in the vicinity of mother trees. As a result, a significant homozygous deficit at roughly 40% of analysed loci along with positive total (0.258) and within-population inbreeding (0.10) has been interpreted as biparental inbreeding due to the local establishment of closely related offspring (Premoli 1996). This would result in the formation of family groups that may in turn be responsible for the elevated genetic diversity found within populations (Premoli 1997).

**Sampling of natural stands**

At different locations within Nahuel Huapi National Park three paired old-growth and postfire stands were selected on the basis of previous information on the disturbance history (Table 1; Kitzberger 1994). Study sites are located in mesic forests at Puerto Blesi (41°01′S, 71°08′W; hereafter Blesi), Lago Espejo (40°31′S, 71°45′W; hereafter Espejo), and Lago Fonck (41°21′S, 71°45′W; hereafter Fonck). At each site one even-aged and one mature stands were established within a radius of < 2 km. Stand origin dates were previously determined by Kitzberger (1994) using dendro-ecological dating of fire scars on partial cross sections of remnant trees. Blesi postfire stand corresponds to the oldest fire c. 10 ha large that occurred in 1917 east of Puerto Blesi.

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Table 1 Stand characteristics of mature and postfire stands of *Nothofagus dombeyi* at three locations within Nahuel Huapi National Park, Argentina. Diameter at breast height (d.b.h.) of remnant trees are indicated as (r)

<table>
<thead>
<tr>
<th>Location</th>
<th>Tree species composition</th>
<th>Density (trees/ha)</th>
<th>d.b.h. X ± SD (cm)</th>
<th>Fire date</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blest</strong></td>
<td>Mature <em>Nothofagus dombeyi</em></td>
<td>96</td>
<td>49.6 ± 52.5</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Postfire <em>Nothofagus dombeyi</em></td>
<td>2786</td>
<td>20.4 ± 6.1</td>
<td>1917</td>
</tr>
<tr>
<td></td>
<td>Postfire <em>Dasyphyllum diacanthoides</em></td>
<td>2143</td>
<td>8.0 ± 3.4</td>
<td>1917</td>
</tr>
<tr>
<td><strong>Espejo</strong></td>
<td>Mature <em>Nothofagus dombeyi</em></td>
<td>257</td>
<td>28.2 ± 21.6</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Postfire <em>Nothofagus dombeyi</em></td>
<td>5306</td>
<td>17.1 ± 9.4</td>
<td>1950</td>
</tr>
<tr>
<td></td>
<td>Postfire <em>Dasyphyllum diacanthoides</em></td>
<td>96</td>
<td>118.0 ± 31.8 (r)</td>
<td></td>
</tr>
<tr>
<td><strong>Fonck</strong></td>
<td>Mature <em>Nothofagus dombeyi</em></td>
<td>148</td>
<td>36.1 ± 43.5</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Postfire <em>Nothofagus dombeyi</em></td>
<td>2943</td>
<td>16.1 ± 14.2</td>
<td>1943</td>
</tr>
<tr>
<td></td>
<td>Postfire <em>Nothofagus antarctica</em></td>
<td>320</td>
<td>12.7 ± 4.8</td>
<td>1943</td>
</tr>
</tbody>
</table>

This stand regenerated at high density early after fire from scattered remnant (c. 35 m tall, 80 cm diameter at breast height (d.b.h.)) Nothofagus dombeyi trees that remain embedded within the even-sized stand (c. 12 m in height, Table 1) today. Later, the shade-tolerant Dasyphllum diacanthoides was massively established underneath the N. dombeyi canopy. Nearby unaffected mature forests c. 300 m away of the fire line is dominated by an N. dombeyi–D. diacanthoides forest with trees up to 35 m in height. Density of adult (> 10 cm d.b.h.) trees is an order of magnitude lower than the nearby postfire stand (Table 1), while seedling/sapling density is much higher. Unlike the other two mature stands, Blst is composed by the shade-intolerant N. dombeyi and the shade-tolerant D. diacanthoides in almost the same density (Table 1). Generally D. diacanthoides pre-empt and dominates gaps caused by N. dombeyi treefalls. In the Espejo postfire stand N. dombeyi established at very high densities after a c. 1200 ha large fire that affected in 1950 the northern end of Lago Espejo. Several remnant trees > 1 m in diameter remain close to the fire line. The mature forest stand located c. 500 m away of the fire line southwards along the eastern shore of Lago Espejo is 20 times less dense than the postfire stand and is composed of a wide variety of sizes from > 70-cm-wide trees to numerous seedlings and saplings. The Fonck postfire N. dombeyi stand colonized massively by seed after a 1943 fire that affected the westernmost end of Lago Fonck whereas the smaller tree Nothofagus antarctica was established by resprouting (Kitzberger 1994). Few remnant surviving trees c. 1 m in diameter remain immersed within the stand. The mature all-sized monspecific N. dombeyi Fonck stand lies on the northern shore of Lago Fonck c. 1.8 km westwards of the 1943 fire.

Stand structure and spatially explicit sampling design
Trees were sampled at each stand within a total area of 1 ha at 10 randomly selected plots of approximately 10 × 10 m. Within each plot a minimum of five trees with d.b.h. > 10 cm were sampled at random for isozyme analysis. The sampling scheme yielded a distribution of distance classes within a total distance of 40 m that included at least 20 pairs of individuals to be analysed at each class in postfire and mature stands. From each individual tree one core was taken by increment borer and diameter was measured at breast height. The position of each tree was mapped to an estimated accuracy of 1 m using X and Y coordinates.

Enzyme extraction and electrophoresis
Fresh leaf tissue was kept cold and transported to the laboratory for protein extraction. Leaves were crushed using liquid nitrogen and the extraction buffer by Mitton et al. (1979). Homogenates were centrifuged and stored in 0.5 mL Eppendorf tubes at −80 °C until electrophoresis on 12% starch gels (Sigma Starch S-4501). Out of several enzyme systems that were previously resolved (Premoli 1994, 1996, 1997), we concentrated on nine loci that produced very clear banding patterns and had sufficiently high levels of polymorphism. A sodium-borate Conkle B buffer system pH 8 (Conkle et al. 1982) run at 40 mA for 6 h was used to resolve phosphoglucoisomerase (Pgi-1, Pgi-2). A morpholine-citrate buffer system pH 7.5 (Ranker et al. 1989) run at 15 mA for 6 h was used to resolve alcohol dehydrogenase (Adh), aldolase (Ald), isocitrate dehydrogenase (Idh), malate dehydrogenase (Mdh-1, Mdh-2, Mdh-3), and shikimate dehydrogenase (Skdh). For each enzyme system, the loci and alleles were numbered sequentially from the most anodal to the most cathodal.

Data analyses
For statistical analyses, different stands (old growth and postfire) at each location were analysed separately. Genetic diversity parameters for each stand were estimated using the program Fpopgene version 1.32 (Yeh et al. 1997): mean number of alleles per locus (A), effective number of alleles (Ae), percent polymorphism sensu stricto (%P), observed heterozygosity (H0), and Nei’s unbiased expected heterozygosity (He). Observed heterozygosity was compared to Hardy–Weinberg (HW) expectations for individual locus and stand using Wright’s (1922) fixation indices (F). Deviations from zero of these indices were tested by chi-squared tests (Li & Horvitz 1953). Differences in gene distributions between stands at each location were evaluated by chi-squared tests (Workman & Niswander 1970). Hierarchical analysis by Wright’s (1965) F-statistics following Weir & Cockerman’s (1984) multilocus estimators and Nei’s (1973) total gene diversity (Ht), were calculated for mature and postfire stands at each location using the program Fstat version 2.9.3.2 by Goudet (2002) (Goudet 1995). F-statistics measure levels of inbreeding within individuals in local stands (Fis), inbreeding due to stand subdivision (Fs), and total inbreeding (Fis). Means and 95% confidence intervals were obtained by resampling schemes on six polymorphic loci. Mean F-statistics were considered significant when confidence intervals did not include zero. Total gene diversity was calculated for each location including mature and postfire stands (Nei 1973).

Spatial genetic structure was analysed by coefficients of spatial autocorrelation: the standard normal deviate (SND) for nominal correlations of genotypes, and Moran’s I for interval data. Spatial analyses were performed using six polymorphic loci (Adh, Ald, Idh, Mdh-2, Pgi-2, and Skdh) along 10 distance classes at 4 m intervals. In the nominal case, the number of joins of every genotype combination for each locus was counted at a given distance class. The nominal autocorrelation test statistic is the standard normal
deviate of the observed number of joins from the expected number of joins (Sokal & Oden 1978):

$$\text{SND} = \frac{\text{Observed joins} - \text{Expected joins}}{\text{Variance}^{1/2}}$$

The SND are assumed to be normally distributed and hence have confidence limits of ± 1.96 at the 5% level of significance. Distance classes were standardized to equalize the number of joins included in each distance class, which influence the variance otherwise. Joins between individuals having identical genotypes are referred to as like joins. Significant ($P < 0.05$) positive SNDs were counted for all like-genotype pairs (LL) as well as the number of joins between like homozygotes (HH) for each locus in each mature and postfire stand. These were estimated using the program \textsc{psawind} version 1.1.1 by Takahashi (2003).

For interval data (such as allele frequencies), Moran’s index $I_q$ measures the degree of spatial autocorrelation (Sokal & Oden 1978; Sokal & Wartenberg 1983):

$$I_q = \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij}(a_i - \bar{a})(a_j - \bar{a})}{\sum_{i=1}^{n}(a_i - \bar{a})^2}$$

$$W = \sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij}$$

when $n$ is the number of samples and $w_{ij} = 1$ if the individuals $i$ and $j$ belong both to the spatial interval $s_q$, otherwise $w_{ij} = 0$. For diploid data, $a_i$ is 1 if the $i$-th individual is homozygous for that allele, 0.5 if heterozygous, and 0 if the individual has no copy of the allele. The value $\bar{a}$ corresponds to the mean value of $a_i$ over all $n$ individuals. Autocorrelation is calculated over all selected loci summing the numerator and denominator of the first equation over the total number of alleles (Streiff et al. 1998). The expected values for the case of no autocorrelation are $-1/(n-1)$ (Sokal & Wartenberg 1983). Higher values indicate positive spatial autocorrelation and smaller values indicate negative spatial autocorrelation. Significant deviation from spatial random distribution was tested by permutation procedures using Monte Carlo simulations. For each spatial distance class, observed values were compared with the distribution obtained after 1000 permutations. Moran’s $I$ were calculated using multilocus estimators by the program \textsc{sos} version 1.0c by Degen (2000) (Degen et al. 2001).

**Results**

**Stand structure**

Frequency distributions of tree ages in age classes of 10 years differ substantially between postfire and mature stands reflecting the dominant modes of regeneration (Fig. 1). All three postfire stands show sharp unimodal age distributions with most ages concentrated right after the age of the fire event. Establishment peaks within the first and second decades after the event and ceases 3–4 decades after the disturbance event. All three mature stands show all-aged frequency distributions with abundant younger
trees and declining numbers of trees in older age classes that reflect relatively continuous regeneration within gaps for at least the last two centuries (Fig. 1).

**Genetic diversity and inbreeding**

Six out of the nine analysed loci were polymorphic in at least one stand. These are Adh, Ald, Idh, Mdh-2, Pgi-2, and Skdh. All loci have banding patterns previously described for *Nothofagus dombeyi* (Premoli 1996, 1997) except Ald, whose pattern coincides to that resolved in *Nothofagus pumilio* (Premoli 2003). In the present study, Adh and Skdh correspond to the previously described polymorphic loci Adh-1 and Skdh-1, respectively (Premoli 1997).

Mean genetic diversity parameters are consistently greater in mature than in postfire stands (Table 2); however, no significant differences in their medians were found (P > 0.2; Mann–Whitney test). All studied locations have comparable average total diversity measured by H_T. Fixation indices were positive (Table 3) and indicated significant (P < 0.05; chi-squared test) deviations to HW equilibrium conditions in 13/19 and 11/17 possible tests in mature and postfire stands, respectively (Table 3). Estimates of fixation indices differed among loci particularly at those with skewed allelic frequencies such as Ald. Hierarchical analysis of inbreeding coefficients yielded mean positive estimates suggesting considerable heterozygous deficiency at most analysed loci and stands. Inbreeding due to subdivision into different stands (F_ST) was significant at Blest (0.076) and Espejo (0.038) suggesting moderate between-stand divergence at those locations (Table 3). This result is also evidenced by the significant (P ≤ 0.05; chi-squared test) between-stand heterogeneity in allelic frequencies at 83%, 83%, and 57% of possible chi-squared tests at Blest, Espejo, and Fonck, respectively.

**Spatial genetic structure**

Out of a total of 95 significant positive SNDSs of like joins for both heterozygote and homozygote pairings, 53 and 41 correspond to mature and postfire stands, respectively. Mature stands concentrate almost 50% of positively like joins within the first three distance classes (< 16 m) whereas postfire stands do so at the fifth distance class (< 24 m). The number of positively like joins is significantly higher in mature stands at distance classes < 16 m (P < 0.05; Mann–Whitney test). Half of the significant positively like joins (27 and 20) correspond to homozygous pairings in mature and postfire stands, respectively. However, mature stands have a similar or greater concentration of homozygous joins than like joins in the first distance classes (Fig. 2). In contrast, postfire stands at Blest in particular and Fonck have higher accumulation of homozygous-like pairings at larger spatial scales (> 24 m) (Fig. 2). However, the postfire stand in Espejo is similar to the corresponding mature stand with an even accumulation of homozygous and like joins throughout distance classes.

Multilocus estimations of Moran’s I yield significant (P < 0.05) autocorrelation coefficients in mature stands at fine spatial scales < 16 m (Fig. 3). Significant correlograms by Moran’s I intercept the abscissa within small spatial distances at Espejo < 16 m and Fonck < 12 m suggesting finescale genetic patchiness. At the Espejo and Fonck mature

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**Table 2** Genetic diversity parameters in mature and postfire stands of *Nothofagus dombeyi* at three locations in northwestern Patagonia, Argentina

<table>
<thead>
<tr>
<th>Location</th>
<th>Stand</th>
<th>N</th>
<th>A</th>
<th>A_e</th>
<th>%P</th>
<th>H_O</th>
<th>H_E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blest</td>
<td>Mature</td>
<td>92</td>
<td>2.44</td>
<td>1.44</td>
<td>0.198</td>
<td>0.231</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.41)</td>
<td>(0.17)</td>
<td>(0.073)</td>
<td>(0.081)</td>
<td></td>
</tr>
<tr>
<td>Postfire</td>
<td>50</td>
<td>2.11</td>
<td>1.30</td>
<td>0.167</td>
<td>0.175</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.39)</td>
<td>(0.14)</td>
<td>(0.105)</td>
<td>(0.069)</td>
<td></td>
</tr>
<tr>
<td>Espejo</td>
<td>Mature</td>
<td>50</td>
<td>2.22</td>
<td>1.39</td>
<td>0.175</td>
<td>0.225</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.32)</td>
<td>(0.15)</td>
<td>(0.069)</td>
<td>(0.073)</td>
<td></td>
</tr>
<tr>
<td>Postfire</td>
<td>61</td>
<td>2.11</td>
<td>1.43</td>
<td>0.158</td>
<td>0.196</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.45)</td>
<td>(0.23)</td>
<td>(0.081)</td>
<td>(0.088)</td>
<td></td>
</tr>
<tr>
<td>Fonck</td>
<td>Mature</td>
<td>50</td>
<td>2.33</td>
<td>1.53</td>
<td>0.213</td>
<td>0.285</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.29)</td>
<td>(0.17)</td>
<td>(0.065)</td>
<td>(0.076)</td>
<td></td>
</tr>
<tr>
<td>Postfire</td>
<td>58</td>
<td>2.44</td>
<td>1.51</td>
<td>0.198</td>
<td>0.282</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.34)</td>
<td>(0.15)</td>
<td>(0.056)</td>
<td>(0.076)</td>
<td></td>
</tr>
</tbody>
</table>

N, sample size; A, mean number of alleles per locus; A_e, effective number of alleles; %P, percent polymorphism; H_O, observed heterozygosity; H_E, expected heterozygosity. Standard errors are in parentheses.

**Table 3** Fixation indices (F) for each locus and stand

<table>
<thead>
<tr>
<th>Loci</th>
<th>Blest</th>
<th>Espejo</th>
<th>Fonck</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mature</td>
<td>Postfire</td>
<td>Mature</td>
</tr>
<tr>
<td>Adh</td>
<td>0.037</td>
<td>-0.719</td>
<td>-0.113</td>
</tr>
<tr>
<td>Ald</td>
<td>-0.022</td>
<td>-0.020</td>
<td>-0.111</td>
</tr>
<tr>
<td>Idh</td>
<td>0.291</td>
<td>0.533</td>
<td>0.168</td>
</tr>
<tr>
<td>Mdh-1</td>
<td>0.656</td>
<td>-0.027</td>
<td></td>
</tr>
<tr>
<td>Mdh-2</td>
<td>0.231</td>
<td>0.441</td>
<td>0.604</td>
</tr>
<tr>
<td>Pgi-2</td>
<td>0.021</td>
<td>0.349</td>
<td>0.201</td>
</tr>
<tr>
<td>Skdh</td>
<td>0.312</td>
<td>0.789</td>
<td>0.411</td>
</tr>
<tr>
<td>Mean</td>
<td>0.145</td>
<td>0.279</td>
<td>0.208</td>
</tr>
</tbody>
</table>

Hierarchical analysis of inbreeding coefficients at each location: F_E is the inbreeding within individuals in local stands, F_GT is the inbreeding due to between-stand divergence at each location, and F_ST is the total inbreeding. H_T is the total genetic diversity at each location. Significance from zero are depicted in bold.
stands Moran’s I values are consistently greater than their postfire counterparts. In contrast, barely significant autocorrelation results were obtained for the mature stand at Blest which also presented Moran’s I values smaller than the corresponding postfire stand. None of the postfire stands show any significant positive deviation in their genetic structure from spatial randomness at any distance (Fig. 3).

Discussion

Stand-level genetic diversity and inbreeding

The effects of regeneration opportunities following treefalls are evident within mature stands of Nothofagus dombeyi which seems to have favoured the maintenance of moderately greater gene diversity in comparison with stands established after coarse-scale fire disturbance. Treefalls in mature stands would allow sporadic gap filling by local regeneration from seeds that are dispersed into distinct spatially and temporally isolated forest openings. However, the genetic make-up of seedlings that establish at any given gap particularly in outcrossing species like N. dombeyi will vary widely with the amount of masting, the number of seed trees, and competition from other species. Nevertheless, our results highlight the importance of old-growth forests as potential reservoirs of genetic diversity which may translate to elevated reproductive capacity as recently demonstrated for red spruce (Mosseler et al. 2003).

In contrast, regeneration of N. dombeyi after coarse-scale disturbances such as fire, often takes place through the establishment of one or a few cohorts of seeds produced during masting events and often by a few surviving adults shortly after fire. Thus, genetic bottlenecks due to a limited number of reproductive trees that regenerate postfire stands will result in a somewhat more genetically uniform progeny. For instance, precise dendrochronological dating of tree establishment dates at the Espejo fire stand indicates that most trees established from a single massive seed cohort produced c. 3 years after the fire, and little further recruitment occurred (Kitzberger 1994). Therefore, age distributions of postfire cohorts within 2–3 decades of establishment may overestimate the regeneration window generated by disturbance at some sites. Potentially, 20–30 years regeneration phases may reflect inevitable dating errors associated with coring large diameter trees in which it is impractical to sample at the root/shoot boundary.

However, differences in diversity parameters between nearby postfire and mature stands were not large. Nothofagus dombeyi is wind pollinated and has, on average, elevated outcrossing rates ranging from 0.87 to 1.04 (Premoli 1996). Thus, coarse-scale disturbances such as fire may leave distinct boundaries beyond which abundant potential parent trees are available for pollination/colonization which may attenuate the effects of genetic bottlenecks during postdisturbance regeneration. Similarly, fire severity may affect the number of remnant trees and therefore, such as in the case of Espejo, several remnant trees regenerated the site and the resulting genetic structure may mimic that of mature stands.

A deficit of heterozygotes was detected at roughly 2/3 of possible tests to equilibrium conditions in all stands. This was greater to that previously estimated (40%) using larger spatial scales for sampling (Premoli 1996). Therefore inbreeding detected by F-statistics at fine spatial scales could be due to a Wahlund effect caused by the local recruitment of related seeds under the vicinity of parent trees. Nothofagus dombeyi seeds are mainly dispersed by gravity and effective seed dispersal measured at postfire edges indicated
that 95% of the seeds fall within 20 m from source trees (Kitzberger 1994). This was particularly evident at Fonck that yielded significant mean $F_{IS}$ caused by generation overlapping. Therefore, neighbouring trees may actually be a mixture of parents and offspring which reinforces genetic structure (Latouche-Hallé et al. 2003), particularly at mature stands. However, the Blest mature stand had a relatively low fixation index. At this stand, the presence of abundant shade-tolerant species like *Dasyphyllum diacanthoides* may inhibit the establishment of *N. dombeyi* seedlings in small openings (Veblen 1985). Thus, recruitment of *N. dombeyi* would seldom occur after gap formation. The resulting progeny at Blest most probably would not share such co-ancestry as in the absence of shade-tolerant competitors (Espejo and Fonck) where synchronous colonization of gaps results in elevated biparental inbreeding and marked genetic structure in mature stands.

Some degree of inbreeding may also occur in *N. dombeyi* (Premoli 1996). Most tree species have elevated inbreeding depression and thus younger trees are expected to have higher fixation indices than older ones. Estimates of inbreeding for mature stands showed that older trees had lower fixation indices ($-0.043$, $0.012$, and $0.064$) than younger individuals ($0.172$, $0.238$, and $0.279$) at Blest, Espejo, and Fonck, respectively. Although based on small sample sizes, this estimate by age class suggests that particularly mature stands with uneven age distribution of individuals appear to combine significant levels of correlated mating among young trees with long-distance gene flow among older individuals. Significant average $F_{ST}$ values along with considerable heterogeneity in allelic frequencies at all studied locations suggest that disturbance throughout drift is maintaining considerable genetic divergence between nearby stands < 5 km away. This is due to the random survival of individual trees that regenerate the stand after fire. However, the density of remnant trees may have an effect on the magnitude of the bottleneck and could explain the heterogeneity in $F_{ST}$ values ranging from $0.076$ at Blest to $0.010$ at Fonck. Although in Blest this may be an artefact due to a larger sample size which may influence the power of tests; it may also reflect the random effects of drift due to low density of remnant trees in this stand that may have accentuated between-stand divergence.

Previously estimated levels of among-population genetic divergence ($G_{ST} \sim F_{ST} = 0.087$) and total diversity ($H_T = 0.224$) on populations of *N. dombeyi* distant 50–230 km from each other (Premoli 1996) are similar to those reported here. Therefore, the stands studied seem to maintain as much genetic diversity as different populations inhabiting different elevations and forest types throughout wider geographical ranges (Premoli 1997). A detailed analysis of genetic structure of *Quercus laevis* yielded elevated average genetic diversity ($H_T = 0.264$) suggesting adequate stand-level variance for appreciable genetic structure (Berg & Hamrick 1995). This also seems to hold for stands of *N. dombeyi* at close proximity which were established in response to disturbances occurring at local spatial scales.
Fine-scale genetic structure

Nearby stands of *N. dombeyi* undergoing different regeneration modes resulted in distinct spatial genetic structures. The standard normal deviate suggests a tendency for a localized clustering in all mature stands at reduced spatial scales < 20 m mimicking the scale of treefalls. A similar pattern was obtained by the spatial autocorrelation analysis particularly for the mature stand at Fonck. Values of average Moran’s *I* are equivalent to twice the values of co-ancestry (Loiselle et al. 1995; Hardy & Vekemans 1999) which has expected values of 0.25 and 0.125 for full- and half-sibs, respectively. Therefore Moran’s *I* values at Espejo and Fonck mature stands (roughly 0.1 and 0.25, respectively) indicate that seedlings within each patch would be at least half-sibs, resulting in significant genetic autocorrelation at short distance classes. In contrast, the mature stand at Blest yielded barely significant autocorrelation results. Therefore, the formation of genetically divergent neighbourhoods as suggested by the spatial autocorrelation analysis (Boyle et al. 1990) has not yet occurred in this stand.

Genetic structure detected in pure mature *N. dombeyi* stands (Fonck and Espejo) could be explained as a result of stand dynamics undergoing gap-phase. *Nothofagus dombeyi* has local seed dispersal and large number of seeds (recruitment attempts) near the parent trees is necessary for successful establishment within nearby gaps. Gaps further away from focal trees are less likely to be colonized by them due to limited dispersal capacity of *N. dombeyi* seeds. These effects together with asynchronous gap formation and shade intolerance of *N. dombeyi* would cause mature stands to generate genetically heterogeneous patches of related propagules at the scale of individual gaps. Strikingly, the weakest genetic structure of mature stands was found at Blest, the only mature stand in this study that had shade-tolerant trees coexisting with *N. dombeyi*. Here, *N. dombeyi* seedlings and saplings have little chances of reaching the overstorey due to the strong competition for light with *D. diacanthoides*. Hence, large emergent *N. dombeyi* can only be explained as the result of past coarse-scale disturbances (old fires, tectonism; Veblen 1989a). Therefore, even if this stand can be considered undergoing gap phase from a community perspective, *N. dombeyi* is composed by an old (genetically homogeneous) postdisturbance cohort producing vain ‘attempts’ of regeneration. This effect, may also explain the lack of differences in fine-scale genetic structure between mature and postfire stands at Blest.

Random distribution of genotypes was detected by autocorrelation in postfire stands within the analysed spatial scale (< 40 m) suggesting that seed shadows may temporally overlap at postfire stands lessening fine genetic substructuring (Hamrick et al. 1993; Hamrick & Nason 1996). We have observed extremely abundant seed production in *Nothofagus* particularly by fire-isolated trees during mast years. On the other hand, the absence of genetic structure may be due to a ‘thinning effect’ during recruitment (Hamrick et al. 1993). Intraspecific competition or random mortality among siblings results in extensive thinning within maternal groups such that only one or a few neighbouring trees will be half-sibs similarly to that found in *Quercus acutissima* (Chung et al. 2002). However, significant SNDs at spatial scales > 30 m in postfire stands at Blest and Fonck may be a reflection of seed dispersal from few highly productive trees that massively and evenly colonize into large openings as a result of fire.

Nevertheless, important differences in fire severity and thus the number of surviving trees were evidenced in postfire stands. These differences may explain why the Espejo postfire stand behaved similarly to the mature counterpart (Fig. 2). In fact, the 1950 fire at Espejo left abundant remnant *N. dombeyi* trees (Mermoz 2005). In contrast, fires at Blest and Fonck were far more severe leaving only a few surviving trees. Therefore, the density of remnant trees may have an important effect on the maintenance of genetic structure which in turn will depend on the severity of fires. Under a global scenario of climate change fires with increasing severity are expected (Kitzberger & Veblen 2003). As a result, few remnant trees will survive and significant losses of genetic structure as well as diversity can be predicted because of genetic bottlenecks during stand recolonization particularly of obligate seed-dispersed and shade-intolerant species such as *N. dombeyi*.

Conclusions

In the southern Andes, the shade-intolerant *Nothofagus dombeyi* builds upon genetically divergent neighbourhoods in response to episodic disturbances. Mature forests seem to consist of a local distribution of genotypes. In contrast, younger even-aged stands established after coarse-scale disturbances are more genetically homogeneous and family groups are eventually found at greater spatial scales. Therefore in mature stands a greater potential exists for the formation of fine genetic structure. These different genetic structures are maintained even in nearby stands within the same landscape.

Under the framework of nonequilibrium theory, ecologists suggested that fine-scale patch dynamics results in complex demographic and genetic structures (Rice & Jain 1985) which remain poorly understood. Models are needed that integrate various processes that promote spatial genetic structures as valuable tools to predict genetic consequences under dynamic scenarios. For example, information on patch size will guide sampling strategies for seed collection from open-pollinated families as well as for mating system and additive genetic variance estimates from progeny tests. It also contributes to predict genetic consequences of forest fragmentation. These models may take into account disturbance regimes along with their spatial
scale, frequency, history, density of remnant reproducing
trees, and distance to seed sources as well as continuous
undisturbed forests. Also they may consider a species auto-
ecological traits including shade tolerance, sprouting ability,
patterns of pollen and seed dispersal together with ger-
mination requirements (e.g. masting, serotiny) and the
development of seed/seedling banks.

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