Spatial genetic structure, population dynamics, and spatial patterns in the distribution of *Ocotea catharinensis* from southern Brazil: implications for conservation

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**Abstract:** In this study, we employ an integrated demographic–genetic approach with the aim of informing efforts to conserve *Ocotea catharinensis* Mez., an endangered tree species from the Brazilian Atlantic Rainforest. After establishing two permanent plots (15 and 15.5 ha) within protected areas in Santa Catarina state, Brazil, we evaluated demographic aspects (density, recruitment, mortality, and growth), spatial pattern, genetic diversity, and spatial genetic structure (SGS) in three categories (seedlings, juveniles, and reproductive individuals) over 2 years. Studied populations presented low recruitment of individuals and low rates of increment in diameter and height. Aggregation was the main spatial pattern observed for both populations. High levels of genetic diversity were estimated for both populations, as well as high levels of fixation index, signaling the risk of losing genetic diversity over generations. Significant SGS was found for both populations, reflecting nonrandom distribution of the genotypes. Demographic and genetic surveys also allowed the estimation of minimum viable areas for genetic conservation (>170 ha), deme sizes (around 10 ha), and distances for seed collection (at least 60 m). Effective population size is restricted in studied populations, locally threatening the species perpetuation over generations. Further research can clarify how this condition will change in subsequent years.

**Key words:** Atlantic Rainforest, fine-scale spatial genetic structure, genetic diversity, neighborhood size, seed collection.

**Résumé :** Nous avons utilisé une approche intégrée de nature génétique et démographique dans le but de fournir des informations pour conserver *Ocotea catharinensis* Mez., une espèce d’arbre menacée de la forêt atlantique brésilienne. Après avoir établi deux parcelles permanentes (15 et 15,5 ha) au sein d’aires protégées dans l’État de Santa Catarina au Brésil, nous avons évalué les aspects démographiques (densité, recrutement, mortalité et croissance), la répartition spatiale, la diversité génétique ainsi que la structure génétique spatiale (SGS) chez trois catégories d’individus (stades de semis, juvénile, et d’âge reproductif) pendant 2 ans. Les populations étudiées présentaient un faible recrutement d’individus et de faibles taux de croissance en diamètre et en hauteur. L’agrégation était le principal patron spatial observé chez les deux populations. Des niveaux élevés de diversité génétique ont été estimés chez les deux populations. Cependant, des niveaux élevés d’indice de fixation ont été observés, signalant un risque de perte de diversité génétique au fil des générations. Une SGS significative a été remarquée chez les deux populations, ce qui reflète une distribution non aléatoire des génotypes. Les études démographique et génétique ont aussi permis d’estimer la superficie minimum viable pour la conservation génétique (>170 ha), la taille des dèmes (environ 10 ha) et la distance pour la collecte de semences (au moins 60 m). La taille effective de population est limitée au sein des populations étudiées, menaçant localement la pérennité de l’espèce au fil des générations. D’autres travaux de recherche pourront clarifier comment cette situation évoluera dans les années à venir. [Traduit par la Rédaction]

**Mots-clés :** forêt atlantique brésilienne, structure génétique spatiale à petite échelle, diversité génétique, taille de déme, récolte de graines.

**Introduction**

Demographic and genetic studies are basic and powerful tools to understanding dynamic processes at the population level and their maintenance over time (Griffith et al. 2016; Lowe et al. 2017). Population structure can be described in terms of the ages, sizes, and forms of the components, and population structure is susceptible to environmental conditions and dynamic processes such as recruitment, mortality, and growth rates, as well as intra- and inter-specific competition (Harper and White 1974). Therefore, demographic studies can help us predict decline, stability, or expansion in populations (Paludo et al. 2016), predict impacts of management practices on the structure of populations (Ribeiro et al. 2014), and unravel environmental and ecological aspects that determine demographic parameters and spatial patterns of distribution (Lara-Romero et al. 2016).

Genetic structure, in turn, results from the spatial nonrandom distribution of genotypes in space (Vekemans and Hardy 2004). At
a more restricted scale (e.g., within a population), genetic structure largely arises from the formation of local pedigrees caused by limited gene flow (Vekemans and Hardy 2004). Life history traits such as regeneration mode (e.g., sprouting or nonsprouting species) plus coarse- and fine-scale disturbances (e.g., gaps, fires, landslides) and particular conditions for seedling establishment can also lead to genetic structuring (Mathiasen and Premoli 2013).

Genetic structure within a population is commonly known as spatial genetic structure (SGS), or fine-scale spatial genetic structure. Studies of SGS and its causes have provided fundamental guidelines for plant conservation and actions, e.g., minimum distances required for seed collections (Tarazi et al. 2010), associations between habitat fragmentation and SGS (Santos et al. 2016), and estimates of neighborhood sizes (Buzatti et al. 2012). By using information gained from both demographic and genetic studies, we might be able to propose feasible management and conservation strategies for plant species.

Ocotea catharinensis Mez. is a tree species that occurs in the Brazilian Atlantic Rainforest (AR) between latitudes 19°57’S (Saitter and Thomaz 2014) and 30°15’S (Carvalho 1994). This species was considered the most abundant and dominant tree in the upper stratum of the Ombrophilous Dense Forest, a forest formation of the AR (Veloso and Klein 1959; Klein 1980) in southern Brazil. However, the intensive exploitation of O. catharinensis to produce timber, combined with the high fragmentation of the AR (Ribeiro et al. 2009; Vibrans et al. 2012), led to the placement of this species on the Brazilian list of endangered plant species (Ministério do Meio Ambiente (MMA) 2014). The species is also classified as vulnerable by the IUCN Red List (Varty and Guadagnin 1998).

Currently, the species occurs at an average density of 5.86 individuals ha⁻¹ (diameter at breast height (dbh) > 10 cm) in remnants of the Ombrophilous Dense Forest in Santa Catarina state (SC) (Lingner et al. 2013); however, in the past, higher densities were recorded, ranging from 23.9 individuals ha⁻¹ (dbh > 12.7 cm) (Veloso and Klein 1959; Klein 1980) to 20 to 50 reproductive individuals ha⁻¹ (Reitz et al. 1978). Densities of 200 to 600 individuals ha⁻¹ higher than 1 m were also reported for SC (Reitz et al. 1978). These major differences between past and present densities call for studies to support conservation efforts regarding O. catharinensis.

Indeed, studies reporting on the demographic aspects of O. catharinensis have already been carried out (Veloso and Klein 1959; Tarazi et al. 2010; Lingner et al. 2013). Nevertheless, such studies have neither covered seedling densities nor focused on population dynamics (e.g., growth rates, mortality, and recruitment). Ocotea catharinensis presents an aggregated spatial pattern (Tarazi et al. 2010); however, the extent of this aggregation, as well as possible variations in the extent and the intensity of the spatial pattern between different categories of individuals (e.g., seedlings, juveniles, or reproductive individuals), are unknown. High levels of genetic diversity, low to moderate levels of fixation index, and moderate genetic divergence are reported for O. catharinensis populations (Tarazi et al. 2010; Martins et al. 2015). Furthermore, significant SGS within populations of O. catharinensis have already been detected (Tarazi et al. 2010). So far, however, genetic diversity and SGS within different categories of individuals are poorly understood.

In this study, we described the population dynamics, spatial pattern, and SGS of three categories of O. catharinensis individuals (seedlings, juveniles, and reproductive individuals) from two populations in SC, southern Brazil. We asked (i) how the demographic structure varies over the years, (ii) how individuals are spatially distributed, and (iii) what is the magnitude of SGS in those populations. This study aimed to provide useful knowledge for O. catharinensis in situ conservation.

### Material and methods

#### Study areas and plots

This study was conducted in two protected areas in SC: Parque Nacional da Serra do Itajai (Serra do Itajai National Park (PNSI), 57 374 ha) and Floresta Nacional de Ibirama (Ibirama National Forest (FNI), 570 ha) (Fig. 1). Vegetation in the study areas is classified as Ombrophilous Dense Forest (ODF), and the climate is described as subtropical humid (Cfa), according to Köppen’s classification. Although currently protected, both areas have previously been subjected to anthropogenic activities at different levels of intensity. For example, selective logging of O. catharinensis was reported for FNI in the 1950s (MMA 2008) and until 1990 for PNSI (MMA 2009). Hunting and illegal exploitation of other key plant species were also reported for both areas (MMA 2008, 2009), and these activities remain a problem (personal observation).

To collect demographic and genetic data, two permanent plots were established: a 15 ha plot (250 m × 600 m) in PNSI and a 15.5 ha plot (350 m × 430 m) in FNI. Two smaller plots subdivided into 10 m × 10 m subplots were installed in the center of the bigger plots (15 and 15.5 ha). These smaller plots comprised 1.9 ha in PNSI (100 m × 190 m) and 1.68 ha in FNI (120 m × 140 m) (Fig. 1). The sampled area in PNSI has an average elevation of 410 m, and vegetation varies from the middle stage of secondary succession on hilltops to the advanced stage on hillsides (MMA 2009). The sampled area in FNI has an average elevation of 350 m and vegetation in the advanced stage of secondary succession (MMA 2008).

#### Demographic characterization

For demographic characterization, Ocotea catharinensis individuals were classified as (i) seedlings, i.e., individuals without measurable dbh, (ii) juveniles, i.e., individuals with measurable dbh up to 20 cm, and (iii) potentially reproductive individuals (hereafter, reproductive), i.e., individuals with dbh > 20 cm. We assessed seedling and juvenile individuals only in the smaller plots and reproductive individuals in both smaller and larger plots. We measured the height of seedlings and juveniles (up to 5 m) and the dbh of juveniles and reproductive individuals over 2 years (2015 and 2016 in PNSI, 2016 and 2017 in FNI). Additionally, we evaluated individual’s mortality, recruitment, density, and increment (height and dbh). Spatial position of each individual was obtained by the xy coordinate system in each of the smaller plots and with GPS (GPSMAP 76CSx, Garmin, Olathe, Kansas, USA) outside the smaller plots.

To visualize the demographic structures in each population, histograms were constructed. The density of individuals was compared between study sites with a 95% confidence interval (95% CI) constructed through bootstrapping 1000 times the density of each category of individuals in each subplot (10 m × 10 m). Confidence intervals (95%) were also constructed for the increment averages of height and dbh (bootstrapped 1000 times). The increment values were obtained through two evaluations in each study site. The CIs were constructed in R (R Core Team 2015).

#### Spatial pattern and spatial independence analysis

Spatial pattern was estimated for all categories of individuals and evaluated years in PNSI and FNI, applying the standardized Ripley’s K function L(r) (Ripley 1981), as implemented in the “spatstat” package (Baddeley and Turner 2005) in R (R Core Team 2015). Spatial independence between seedlings and reproductive individuals was tested using the bivariate extension of Ripley’s K_{LZ}(r) (Lotwick and Silverman 1982), as implemented in the “spalansc” package (Rowlingson and Diggle 2015) in R (R Core Team 2015). For both analyses, we used a radius (r) of 1 m to estimate L(r) and I_{LZ}(r), and the spatial pattern was evaluated up to half of the smaller dimension of the plots, or up to 50 m and 60 m for smaller PNSI and FNI plots (seedlings and juveniles), respectively, and up to 175.5 m and 125 m for larger PNSI and FNI plots (reproductive),
respectively. Deviations from complete spatial randomness were tested through a 95% CI obtained from 1000 Monte Carlo simulations of completely random events.

Genetic data acquisition and analysis

At both study sites, fresh leaves were collected from all O. catharinensis individuals occurring in the smaller plots plus all reproductive individuals occurring in the larger plots. Furthermore, individuals that recruited into seedling category were also sampled by the second demographic evaluation (2016 in PNSI and 2017 in FNI). Genetic characterization was performed using allozyme markers, which have been widely used for several studies reporting on population genetics, including SGS (Vekemans and Hardy 2004; Tarazi et al. 2010; Quipildor et al. 2017). Enzymes were extracted by macerating the leaf tissue for 15 s with an automatic homogenizer (6500 rotations·min⁻¹).

The following enzymatic systems were resolved in starch gel (Penetrose 30%–13%), using a Tris–Citrate pH 7.5 buffer (Tris, 27 g·L⁻¹; citric acid, 16.52 g·L⁻¹) for electrophoresis: aspartate transaminase (at, EC 2.6.1.1), diaphorase (dia, EC 1.8.1.4), esterase (est, EC 3.1.1.1), glucose-6-phosphate dehydrogenase (g6pdh, EC 1.1.1.49), glutamate dehydrogenase (gdh, EC 1.4.1.2), isocitrate dehydrogenase (idh, EC 1.1.1.42), malic enzyme (me, EC 1.1.1.40), malate dehydrogenase (mdh, EC 1.1.1.37), and phosphoglucomutase (pgm, EC 5.4.2.2).

To characterize the genetic diversity of populations and their categories, we estimated the following indexes: percentage of polymorphic loci ($\hat{P}$), total number of alleles ($\hat{k}$), average number of alleles per locus ($\bar{A}_n$), observed ($\hat{H}_O$) and expected ($\hat{H}_E$) heterozygosity, and fixation index ($\hat{f}$). Effective population size ($\hat{N}_e$, the size of an idealized population that would have the same amount of inbreeding as the population under consideration; Kimura and Crow 1963) was estimated following the equation proposed by Li (1976): $\hat{N}_e = n(1 + \hat{f})$, where $n$ is the number of sampled individuals. Statistical significance ($p < 0.05$) for $\hat{f}$ values was obtained through 1000 permutations of alleles among individuals. All analyses were carried out in FSTAT software (version 2.9.3.2; Goudet 2002), except for $\hat{A}_E$ and $\hat{N}_e$. Based on the $\hat{N}_e$, we estimated the minimum viable areas (mva) for long-term genetic conservation as follows: $\text{mva} = \frac{\hat{N}_e}{\hat{f}} \cdot \bar{d}$, where $\hat{N}_e$ is a reference value of 1000 individuals, which was proposed by Lynch (1996) as sufficient to mitigate the effects of deleterious mutations, $n$ is the sample size, and $\bar{d}$ is the density of reproductive individuals in each population. These estimates were made by taking into account only reproductive individuals in the last year of evaluation in each population (2016 in PNSI and 2017 in FNI).

Spatial genetic structure (SGS) for both populations and all categories was estimated by performing an autocorrelation analysis using the coancestry coefficient ($\hat{\theta}_{xy}$), as described in Loiselle et al. (1995). We established equal distance classes for both populations (20 m) to facilitate comparisons, observing a minimum of 30 pairs of observations in each class. To test the statistical significance of $\hat{\theta}_{xy}$ within each distance class, a 95% CI was constructed by 10 000 permutations of individual locations among all individuals. SGS analysis was performed using SPAGeDi software (version 1.5; Hardy and Vekemans 2002).

Fig. 1. Map showing both study sites and details of permanent plots.
Neighborhood size ($N_b$, the effective population number in an area from which the parents may be assumed to be drawn at random; Wright 1940) for reproductive individuals within each studied population was estimated as $N_b/H_1H_2^{1/b_k}$ (Vekemans and Hardy 2004). In this formula, $H_1$ is the ancestry coefficient in the first class of distance (0–20 m), and $b_k$ is the regression slope of the ancestry coefficient value on the logarithm of spatial distance between individuals, but within plot distances, as follows: 0 to 560 m in PNSI and 0 to 480 m in FNI. $N_b$ was estimated using SPAGeDi (version 1.5; Hardy and Vekemans 2002). Deme sizes (ha) were estimated for each studied population based on $N_b$ values, as in the following equation: deme size = $N_b/d$, where $d$ is the density of reproductive individuals in each population.

### Results

#### Population structure and dynamics

The populations presented similar densities of juveniles and reproductive individuals, but PNSI presented statistically higher density of seedlings (Table 1). Recruitments in the seedling category were recorded for both populations (PNSI = 5 individuals; FNI = 1 individual), as well as recruitment of seedlings into the juvenile category (PNSI = 8 individuals; FNI = 11 individuals) (Table 1). Mortality was verified only for seedlings from PNSI (9 individuals) (Table 1). Distribution of individuals per dbh class presented an inverted J-shaped form for both populations; however, PNSI showed a gap of individuals in the 10 cm to 20 cm and 20 cm to 30 cm dbh classes (Fig. 2).

#### Increment averages in height and diameter at breast height

Increment averages in height and diameter at breast height (dbh) were similar between populations and categories, except for seedlings from FNI, which showed statistically higher height increment compared with seedlings from PNSI (Table 2). All increment averages presented in Table 2 represent confidence intervals (95%) for increment averages.

### Table 1. Demographic estimates in two populations of Ocotea catharinensis (PNSI and FNI) from the Atlantic Rainforest, Santa Catarina, Brazil.

<table>
<thead>
<tr>
<th>Category</th>
<th>Individuals</th>
<th>Density</th>
<th>recr</th>
<th>dead</th>
<th>nf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedlings</td>
<td>217</td>
<td>198</td>
<td>114.2 (90.5/142.6)</td>
<td>104.2 (81/131.1)</td>
<td>5</td>
</tr>
<tr>
<td>Juveniles</td>
<td>59</td>
<td>67</td>
<td>31.1 (22.6/39.5)</td>
<td>35.3 (26.3/45.2)</td>
<td>8</td>
</tr>
<tr>
<td>Reproductive</td>
<td>75</td>
<td>75</td>
<td>5 (3.8/6.2)</td>
<td>5 (3.8/6.2)</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>Individuals</th>
<th>Density</th>
<th>recr</th>
<th>dead</th>
<th>nf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedlings</td>
<td>51</td>
<td>41</td>
<td>30.4 (19/42.3)</td>
<td>24.4 (16.1/32.7)</td>
<td>1</td>
</tr>
<tr>
<td>Juveniles</td>
<td>52</td>
<td>63</td>
<td>31 (22/39.9)</td>
<td>37.5 (27.4/48.2)</td>
<td>11</td>
</tr>
<tr>
<td>Reproductive</td>
<td>63</td>
<td>63</td>
<td>4.1 (3.3/5.2)</td>
<td>4.1 (3.3/5.2)</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: PNSI, Parque Nacional da Serra do Itajaí; FNI, Floresta Nacional de Ibirama; individuals, total number of individuals in each category; density, number of individuals per hectare in each category; recr, number of recruitments; dead, number of dead individuals; nf, number of individuals not found. Values in parentheses represent confidence intervals (95%) for density averages.

### Table 2. Increment averages in height and diameter at breast height (dbh) in two populations of Ocotea catharinensis (PNSI and FNI) from the Atlantic Rainforest, Santa Catarina, Brazil.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Category</th>
<th>Increment</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNSI</td>
<td>Height (m) Seedlings</td>
<td>0.07 (0.05/0.08)</td>
<td>0.10</td>
<td>0.64</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Height (m) Juveniles</td>
<td>0.14 (0.09/0.2)</td>
<td>0.17</td>
<td>0.68</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dbh (cm) Juveniles</td>
<td>0.12 (0.08/0.18)</td>
<td>0.20</td>
<td>0.80</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dbh (cm) Reproductive</td>
<td>0.8 (0.58/1.02)</td>
<td>0.48</td>
<td>1.60</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>FNI</td>
<td>Height (m) Seedlings</td>
<td>0.13 (0.09/0.15)</td>
<td>0.11</td>
<td>0.55</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Height (m) Juveniles</td>
<td>0.13 (0.07/0.19)</td>
<td>0.16</td>
<td>0.70</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dbh (cm) Juveniles</td>
<td>0.15 (0.09/0.2)</td>
<td>0.18</td>
<td>0.60</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dbh (cm) Reproductive</td>
<td>1.04 (0.76/1.33)</td>
<td>1.00</td>
<td>3.80</td>
<td>51</td>
<td></td>
</tr>
</tbody>
</table>

Note: PNSI, Parque Nacional da Serra do Itajaí; FNI, Floresta Nacional de Ibirama; SD, standard deviation; Min, minimum; Max, maximum; n, number of individuals. Values in parentheses represent confidence intervals (95%) for increment averages.
high associated standard deviations (SD), evidencing the heterogeneity of these features in the studied populations (Table 2).

**Spatial pattern and spatial dependence**

Aggregation, with different intensities and at different distances, was the main spatial pattern observed in both studied populations and their categories (Fig. 3). Seedling individuals from PNSI presented aggregation from 0 m to 50 m in 2015 and 2016. Juveniles were aggregated from ≈5 to 50 m in 2015 and 2016, and reproductive individuals presented aggregation from ≈5 to 125 m (2015 and 2016) (Fig. 3). In FNI, seedlings presented an aggregated spatial pattern from 0 m to 60 m in 2016 and from ≈5 m to ≈18 m in 2017. Juveniles presented no aggregation in 2016, but aggregation from 0 m to ≈8 m was recorded in 2017. Reproductive individuals were aggregated from ≈2 m to ≈75 m (2016 and 2017) (Fig. 3). Aggregation intensity (L(r)) was higher for individuals from PNSI compared with FNI (Fig. 3). Seedlings and juveniles from PNSI and FNI presented spatial distribution independent of the spatial distribution of reproductive individuals (Fig. 4).

**Genetic diversity**

Between PNSI and FNI, 477 individuals were genotyped, and 21 different alleles were recorded for both populations (Table 3). The number of individuals composing each category followed the results of the demographic survey. For instance, seedlings that recruited into the juvenile category (PNSI = 8 individuals; FNI = 11 individuals) were analyzed as seedlings in the first year and as juveniles in the second year. Average number of effective alleles per locus (ÂE) represented about 70% of Â for both populations, indicating the occurrence of common alleles on the assessed loci. Diversity indexes were quite similar between years, categories, and populations (Table 3). Significant deviations from Hardy–Weinberg equilibrium were detected for seedlings and reproductive individuals from PNSI and FNI, indicating a deficit of heterozygotes (Table 3). Effective population size was around 13% smaller than sample sizes, reflecting the Šest estimates. Minimum viable areas, taking a reference size of 1000 individuals (Lynch 1996), were equal to 176 ha and 205 ha for PNSI and FNI, respectively.
Spatial genetic structure

Significant SGS was found for both studied populations. Seedlings from PNSI presented positive and significant coancestry (\( \hat{\theta}_{xy} \)) values when separated by less than 40 m in both evaluated years. Juveniles from PNSI did not show significant SGS, while reproductive individuals from PNSI presented positive and significant \( \hat{\theta}_{xy} \) values in the first and third distance classes (0–20 m and 40–60 m) (Fig. 5). For FNI, positive and significant \( \hat{\theta}_{xy} \) values were detected for seedlings (2016 and 2017) in the first distance class (0–20 m). Juveniles also presented positive and significant \( \hat{\theta}_{xy} \) values in the first distance class (0–20 m), but only in 2017. For reproductive individuals, no significant SGS was recorded (Fig. 5). All significant

Table 3. Genetic estimates for two populations of Ocotea catharinensis (PNSI and FNI) from the Atlantic Rainforest, Santa Catarina, Brazil.

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>( \hat{N}_e )</th>
<th>( \hat{P} )</th>
<th>( \hat{k} )</th>
<th>( \hat{A} )</th>
<th>( \hat{A}_e )</th>
<th>( \hat{H}_E )</th>
<th>( \hat{H}_O )</th>
<th>( \hat{f} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PNSI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seedlings 2015</td>
<td>196</td>
<td>172</td>
<td>63.6</td>
<td>21</td>
<td>1.91</td>
<td>1.43</td>
<td>0.223</td>
<td>0.191</td>
<td>0.143*</td>
</tr>
<tr>
<td>Seedlings 2016</td>
<td>193</td>
<td>168</td>
<td>63.6</td>
<td>21</td>
<td>1.91</td>
<td>1.43</td>
<td>0.223</td>
<td>0.190</td>
<td>0.148*</td>
</tr>
<tr>
<td>Juveniles 2015</td>
<td>56</td>
<td>52</td>
<td>63.6</td>
<td>20</td>
<td>1.82</td>
<td>1.40</td>
<td>0.224</td>
<td>0.204</td>
<td>0.091</td>
</tr>
<tr>
<td>Juveniles 2016</td>
<td>64</td>
<td>60</td>
<td>63.6</td>
<td>20</td>
<td>1.82</td>
<td>1.39</td>
<td>0.222</td>
<td>0.207</td>
<td>0.067</td>
</tr>
<tr>
<td>Reproductive 2015–2016</td>
<td>68</td>
<td>60</td>
<td>63.6</td>
<td>20</td>
<td>1.82</td>
<td>1.34</td>
<td>0.196</td>
<td>0.171</td>
<td>0.131*</td>
</tr>
<tr>
<td>All 2015</td>
<td>320</td>
<td>279</td>
<td>63.6</td>
<td>21</td>
<td>1.91</td>
<td>1.42</td>
<td>0.221</td>
<td>0.189</td>
<td>0.147</td>
</tr>
<tr>
<td>All 2016</td>
<td>325</td>
<td>283</td>
<td>63.6</td>
<td>21</td>
<td>1.91</td>
<td>1.42</td>
<td>0.222</td>
<td>0.189</td>
<td>0.146</td>
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<tr>
<td><strong>FNI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Seedlings 2016</td>
<td>51</td>
<td>44</td>
<td>63.6</td>
<td>20</td>
<td>1.82</td>
<td>1.37</td>
<td>0.206</td>
<td>0.177</td>
<td>0.139*</td>
</tr>
<tr>
<td>Seedlings 2017</td>
<td>41</td>
<td>35</td>
<td>63.6</td>
<td>20</td>
<td>1.82</td>
<td>1.39</td>
<td>0.213</td>
<td>0.179</td>
<td>0.165*</td>
</tr>
<tr>
<td>Juveniles 2016</td>
<td>52</td>
<td>47</td>
<td>72.7</td>
<td>21</td>
<td>1.91</td>
<td>1.39</td>
<td>0.214</td>
<td>0.193</td>
<td>0.099</td>
</tr>
<tr>
<td>Juveniles 2017</td>
<td>63</td>
<td>58</td>
<td>72.7</td>
<td>21</td>
<td>1.91</td>
<td>1.37</td>
<td>0.208</td>
<td>0.191</td>
<td>0.084</td>
</tr>
<tr>
<td>Reproductive 2016–2017</td>
<td>63</td>
<td>53</td>
<td>63.6</td>
<td>20</td>
<td>1.82</td>
<td>1.36</td>
<td>0.204</td>
<td>0.168</td>
<td>0.177*</td>
</tr>
<tr>
<td>All 2016</td>
<td>165</td>
<td>144</td>
<td>72.7</td>
<td>21</td>
<td>1.91</td>
<td>1.37</td>
<td>0.208</td>
<td>0.178</td>
<td>0.143</td>
</tr>
<tr>
<td>All 2017</td>
<td>166</td>
<td>146</td>
<td>72.7</td>
<td>21</td>
<td>1.91</td>
<td>1.37</td>
<td>0.208</td>
<td>0.179</td>
<td>0.141</td>
</tr>
</tbody>
</table>

Note: PNSI, Parque Nacional da Serra do Itajaí; FNI, Floresta Nacional de Ibirama; n, sample size; \( \hat{N}_e \), effective population size; \( \hat{P} \), total number of alleles; \( \hat{k} \), percentage of polymorphic loci; \( \hat{A} \), average number of alleles per locus; \( \hat{A}_e \), average number of effective alleles per locus; \( \hat{H}_E \), expected heterozygosity; \( \hat{H}_O \), observed heterozygosity; \( \hat{f} \), fixation index; *, \( p < 0.05 \).
and positive coancestry values ranged from 0.037 (PNSI seedlings in 2015) to 0.073 (FNI seedlings in 2016). Negative and significant \( \hat{\theta}_{xy} \) values were verified for reproductive individuals in the distance classes of 140 m to 160 m and 180 m to 200 m in PNSI and FNI, respectively (Fig. 5).

Neighborhood size estimates (\( \hat{N}_b \)) for reproductive individuals from PNSI and FNI were equal to 44 and 48 individuals, respectively. Under the estimated densities of reproductive individuals (Table 1), deme sizes were estimated to be 8.8 ha and 11.7 ha for PNSI and FNI, respectively.

**Discussion**

This is the first attempt to understand demographic and genetic aspects of *O. catharinensis* over more than just 1 year. Nevertheless, it is important to keep in mind that *O. catharinensis* is a long-lived species, well distributed across the Ombrophilous Dense Forest. Moreover, all of the presented results and the following discussions are based on a 2-year survey (genetic and demographic) in two populations. For that reason, our data and discussions should be interpreted considering the particularities mentioned.

**Population structure and dynamics**

Both studied populations presented densities of reproductive individuals (Table 1) compatible with those of the most recent study. *Ocotea catharinensis* occurs at an average density of 5.86 individuals·ha\(^{-1}\) (dbh > 10 cm) in remnants of the Ombrophilous Dense Forest in SC (data from 197 sample units) (Lingner et al. 2013). Nevertheless, estimated densities for both studied populations (Table 1) were found to be substantially lower than estimated densities for *O. catharinensis* populations in the past. In SC, densities of 23.9 individuals·ha\(^{-1}\) (dbh > 12.7 cm) were previously estimated in eight sample units (Veloso and Klein 1959). Additionally, observations by Reitz et al. (1978) reported densities ranging from 20 to 50 reproductive individuals·ha\(^{-1}\) and from 200 to 600 individuals·ha\(^{-1}\) higher than 1 m. Such discrepancy between past (Veloso and Klein 1959; Reitz et al. 1978) and present (Tarazi et al. 2010; Lingner et al. 2013; Table 1) estimates can be attributed to the intensive exploitation of *O. catharinensis* for timber in the intervening years. *Ocotea catharinensis* is considered one of the three most exploited timber species in the Ombrophilous Dense Forest, supplying basically the national market (Reitz et al. 1978).
Recruitments into the seedling category occurred at lower intensity than mortality of seedlings for the PNSI population, and for the FNI population, only one seedling individual was recruited (Table 1). Corroborating our results concerning increment rates (Table 2), O. catharinensis is ranked as one of the three species with lowest growth rate among 100 tree species from Brazil, as listed by Carvalho (1994). These results demonstrate that natural reestablishment of past densities could be a slow process. For instance, at the estimated average of dbh increment for juveniles from FNI (Table 2), an individual with dbh = 1 cm could be expected to reach a dbh = 20 cm in about 126 years (95% CI = 95–211 years).

The distribution of individuals per dbh classes (Fig. 2) presented an inverted J-shaped form for both populations, demonstrating that the studied populations still present the potential for replacement of larger individuals, even with the low intensity of seedling recruitment (Table 1). A gap of individuals was observed in the 10 cm to 20 cm and 20 cm to 30 cm dbh classes for the PNSI population (Fig. 2), owing to logging activities reported for PNSI until 1990 (MMA 2009). Therefore, these gaps may be the result of past exploitation processes, reducing the regeneration capacity of the population by the withdrawal of reproductive individuals.

Spatial pattern and spatial independence
An aggregated distribution pattern is predominant for tropical tree species, resulting from heterogeneous environments (Condit et al. 2000). This aggregated distribution pattern was also recorded for O. catharinensis (Tarazi et al. 2010) and O. porosa (Canalez et al. 2006). The studied populations presented mostly aggregated distribution, with the exception of juveniles from FNI (Fig. 3). In general, aggregation intensity ($I(r)$) was higher for individuals from PNSI, suggesting higher environmental heterogeneity in this study site compared with FNI (Fig. 3). As mentioned, the stage of secondary succession in the PNSI plot varies according to the elevation (middle stage on hilltops and advanced stage on hill-sides; MMA 2009), while in the FNI plot, the vegetation only presents the advanced stage of secondary succession (MMA 2008).

Comparing both studied populations, it is possible to observe that spatial distribution of seedlings and juveniles was affected by mortality and recruitment. Seedlings from PNSI presented a more intense aggregation in 2016 than in 2015; on the other hand, seedlings from FNI were aggregated from 0 m to ≈ 8 m in 2017 (Fig. 3). Recruitment of seedlings into the next category caused a slight decrease in the aggregation intensity of juveniles in PNSI. Nevertheless, the opposite result can be seen for FNI juveniles, which were not aggregated in 2016 and presented aggregation from 0 m to ≈ 8 m in 2017 (Fig. 3).

Spatial distribution of seedlings was independent of the spatial distribution of reproductive individuals (Fig. 4). Therefore, spatial distribution of seedlings is unlikely to be associated to the barochory reported for O. catharinensis (Moraes and Paoli 1995). The better germination of seeds in conditions of higher soil humidity (Moraes and Paoli 1999), combined with high rates of deteriorated seeds found under seed trees (Moraes and Paoli 1995, 1999), indicating density-dependent recruitment, are factors that could better explain the aggregation and spatial independence of seedlings. The action of fauna dispersing seeds can also influence the spatial pattern and independence of seedlings. For instance, Brachyteles arachnoides, one of the dispersers of O. catharinensis (Moraes and Paoli 1995), presents two types of behavior when feeding on trees of Cryptocarya moschata (Lauraceae): (i) feeding from only one seed tree, dispersing seeds in suitable places for seedling recruitment (Moraes et al. 1999), which favors aggregation and spatial independence, and (ii) feeding from several seed trees, dispersing seeds along the area (Moraes et al. 1999), which favors random distribution.

A comparison of PNSI and FNI based on differences in spatial distribution between juveniles and reproductive individuals (Fig. 3) can be viewed as a reflection of the stage of secondary succession of each study site. In the case of reproductive individuals, spatial distribution can also be influenced by past logging activities reported for both populations (MMA 2008, 2009). Nevertheless, it is a difficult task to evaluate such influence, essentially because evidence of exploited individuals such as rotten logs has, of course, long since vanished.

Genetic diversity
Tree species of the Lauraceae family apparently trend toward high levels of genetic diversity (Chung et al. 2003; Tarazi et al. 2010; Reis et al. 2012; Martins et al. 2015). The estimated genetic diversity ($H_e$) for each population (PNSI = 0.222; FNI = 0.208) can also be considered high when compared with average $H_e$ for long-lived perennial woody species (0.149) (Hamrick and Godt 1989). This is an important result for the conservation of O. catharinensis, highlighting the role of protected areas in the conservation of genetic diversity of several species and demonstrating that PNSI and FNI populations present the real potential for conservation.

Significant and positive deviations from Hardy–Weinberg equilibrium were also recorded, on average, for other populations of O. catharinensis (Reis et al. 2012; Martins et al. 2015) and for the congeneric species O. porosa and O. odorifera (Reis et al. 2012). Nevertheless, significant excess of heterozygotes were also recorded for populations of O. catharinensis (Tarazi et al. 2010). Several factors can be influencing these divergences, e.g., more or less intense historical exploitation of each evaluated fragment, SGS levels in each population, which can affect genetic estimates (Jones and Hubbell 2006), biparental inbreeding, and sampling strategies of each study. However, given a single reproduction event under Hardy–Weinberg equilibrium, the expected fixation index of seedlings should be zero for an outcrossing species (Hartl and Clark 2007). Ocotea catharinensis is presumably an outcrossing species, presenting an apparent outcrossing rate of 1.0 (Tarazi et al. 2010); however, aspects of its reproductive biology such as mating system and pollination ecology are still not fully understood. Future studies to elucidate the reproductive system of the species could clarify this question.

Positive and significant estimates of fixation indexes ($f$) were recorded for reproductive individuals from both studied populations, and these estimates resulted in effective sizes ($N_e$) reductions (Table 3). Based on $N_e$ and taking a reference size of 1000 individuals (Lynch 1996), minimum viable area estimates (176 ha for PNSI and 205 ha for FNI) were smaller than the total areas at the study sites (PNSI = 57 374 ha; FNI = 570 ha). Thus, both study sites are able to conserve large populations, even larger than 1000 individuals. Populations lose genetic diversity at a rate of $1/2N$, where $N$ is the number of individuals (Wright 1931); therefore, small populations will lose genetic diversity faster than larger populations. This result demonstrates the potential importance of fragment size in mitigating the effects of fixation indexes.

Spatial genetic structure
Significant SGS has been reported for a number of tropical and neotropical tree species (Hardy et al. 2006) and also for trees of the Lauraceae family such as Cryptocarya moschata (Moraes et al. 2004). Nevertheless, no significant SGS was observed for other trees of Lauraceae, for instance, O. odorifera (Kageyama et al. 2003) and Cinnamomum insularimontanum (Chung et al. 2003). These differences among species can be influenced by environmental conditions but also by aspects related to the reproductive biology and gene flow of each species (Chung et al. 2003; Vekemans and Hardy 2004; Hardy et al. 2006).

Ocotea catharinensis presented significant SGS up to distances of 80 m (Tarazi et al. 2010). Our SGS results for reproductive individuals differ from those found by Tarazi et al. (2010). Reproductive individuals from FNI lack SGS, while reproductive individuals from PNSI present significant SGS up to 60 m, except in the distance class from 20 m to 40 m (Fig. 5). These differences can be...
explained in several ways: (i) methodological approaches — our reproductive category included only individuals withdbh > 20 cm, while Tarazi et al. (2010) sampled individuals with dbh > 6.85 cm; (ii) environmental conditions of each study site — this could, for example, include the presence (or absence) and abundance of seed dispersers; and (iii) historical exploitation of each study site — both PNSI and FNI have undergone adverse logging activities (MMA 2008, 2009). Selective logging may reduce the distance of the SGS for reproductive individuals such as that observed for *Hymenaea courbaril* (Lacerda et al. 2008).

Significant SGS up to 40 m for PNSI and up to 20 m for FNI was estimated for seedlings (Fig. 5). As discussed, spatial distribution of seedlings is independent of the spatial distribution of reproductive individuals for both populations (Fig. 4); therefore, barochory cannot explain the SGS of seedlings. *Brachyteles arachnoides*, one of the dispersers of *O. catharinensis* (Moraes and Paoli 1995), presented two types behavior when feeding on the Lauraceae tree *Cryptocarya moschatu*, as previously mentioned. Hence, the preference for germination in conditions of higher soil humidity (Moraes and Paoli 1999), the high rates of deteriorated seeds found under seed trees (Moraes and Paoli 1995, 1999), and the behavior of seed dispersers are all factors that can explain the SGS of seedlings, apart from barochory.

The mortality and recruitment of seedlings and juveniles resulted in slight changes in SGS patterns of these categories in FNI (Fig. 5). The recruitment of 11 seedlings into the juvenile category in 2017 (Table 1) led to the appearance of significant SGS in the first distance class for juveniles (0–20 m) and the reduction of the SGS level in the first distance class for seedlings (0–20 m) (Fig. 5). The same trend was not observed for seedlings and juveniles from PNSI, possibly because seedling density in this population is larger than that in the FNI population (Table 1). Consequently, the processes of recruitment and mortality represent only a small portion of total density. An increase in SGS in older cohorts relative to younger cohorts can result from several factors such as bottleneck or founder effects, local adaptation owing to microhabitat selection, limited dispersal, and more overlapping generations in older cohorts compared with younger cohorts (Jones and Hubbell 2006).

Neighborhood size estimates ($\bar{N}$) (PNSI = 44 individuals and FNI = 48 individuals) can be considered intermediate when compared with other estimates for the species (25 to 83 individuals: Tarazi et al. 2010). Nevertheless, under the estimated densities of reproductive individuals (Table 1), estimates of deme size in the present study (PNSI = 8.8 ha and FNI = 11.7 ha) were greater than those of Tarazi et al. (2010) at 5 to 6 ha. These estimates can be interpreted as a minimum required area for conservation of a deme.

**Implications for conservation**

Estimated population densities by the present study and other recent studies (Tarazi et al. 2010; Lingner et al. 2013) are considerably lower than historical densities (Veloso and Klein 1959; Reitz et al. 1978), reflecting the intensive timber exploitation. Furthermore, results of our demographic survey revealed a low recruitment of new individuals into populations, as well as low rates of increment in dbh and height. Therefore, the negative effects caused by exploitation on the demographic aspects of this species may require a protracted period of recovery. In this sense, it is important to continue studying population dynamics of *O. catharinensis*, especially to better understand the factors influencing the low recruitment of seedlings. Supra-annual seed production limited to a few seed trees was observed for *O. catharinensis* (Silva et al. 2000), and this can be a factor influencing the regenerative potential of the species. It is also important to continue restricting exploitation of *O. catharinensis* to avoid further reductions in population sizes.

As part of our integrative approach, the genetic survey returned valuable information regarding conservation of genetic diversity. Large areas are imperative for long-term conservation of the species — ideally greater than 170 ha, according to the minimum viable areas estimated. Selecting large areas to conserve *O. catharinensis* populations might present other advantages such as high probability of the presence of fauna, an important vector of seed dispersal, and high occurrence of adequate microhabitats for seed germination and seedling establishment. Nevertheless, deme sizes were estimated to be around 10 ha for both PNSI and FNI, signaling that small fragments can also be important in the genetic conservation of *O. catharinensis*. It should be noted that AR is currently highly fragmented (Ribeiro et al. 2009; Vibrans et al. 2012), and in this sense, small fragments can harbor rare and exclusive alleles and could also serve as binding elements between larger fragments.

Regarding ex situ conservation, SGS analysis revealed two important distances to be respected to avoid collection of seeds from closely related individuals. To minimize the probability of sampling closely related individuals, it is recommended to sample seeds of individuals separated by at least 60 m for PNSI and 40 m for FNI. The FNI population did not present significant SGS, but a tendency toward structuration up to 40 m was observed (Fig. 5). To maximize the capture of genetic diversity, seeds should be sampled from seed trees separated by 140 m to 160 m in PNSI and 180 m to 200 m in FNI. These ranges represent the distance at which reproductive individuals presented negative and significant estimates of coancestry (Fig. 5); therefore, these are also ranges at which individuals are more genetically distinct.

The major differences between present and past estimates of density and the high levels of fixation indexes for seedlings are signaling major restrictions of effective population size caused by the intense timber exploitation. This condition summed up with the low rates of growth and recruitment can imply a loss of genetic diversity and population dynamism over generations, thereby locally threatening the species perpetuation. Thus, it is important to continuously monitor and study *O. catharinensis* populations to verify whether this condition is unique to the studied populations and how it will change in subsequent years.

Finally, the conclusions above were made possible through the integration of demographic and genetic data and the evaluation of demographic and genetic aspects of two *O. catharinensis* stands over a 2-year period, highlighting the importance of such an integrative approach for studies of endangered tree species. Although *O. catharinensis* is a longevous species, this is the first attempt to understand its demographic and genetic aspects over more than 1 year.

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