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<td>cjfr-2017-0446.R1</td>
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<td>Manuscript Type:</td>
<td>Article</td>
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<td>Date Submitted by the Author:</td>
<td>05-Feb-2018</td>
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<td>Complete List of Authors:</td>
<td>Montagna, Tiago; Universidade Federal de Santa Catarina, Fitotecnia Lauterjung, Miguel; Universidade Federal de Santa Catarina, Fitotecnia Candido-Ribeiro, Rafael; Universidade Federal de Santa Catarina, Fitotecnia Silva, Juliano; Universidade Federal de Santa Catarina, Fitotecnia Hoeltgebaum, Marcia; Universidade Federal de Santa Catarina, Fitotecnia Costa, Newton; Universidade Federal de Santa Catarina, Fitotecnia Bernardi, Alison; Universidade Federal de Santa Catarina, Fitotecnia Reis, Mauricio; Universidade Federal de Santa Catarina, Fitotecnia</td>
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<td>Keyword:</td>
<td>Atlantic Rainforest, fine-scale spatial genetic structure, genetic diversity, neighborhood size, seed collection</td>
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<td>Is the invited manuscript for consideration in a Special Issue? :</td>
<td>N/A</td>
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https://mc06.manuscriptcentral.com/cjfr-pubs
Spatial genetic structure, population dynamics and spatial patterns in the distribution of *Ocotea catharinensis* Mez. 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*, 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 of individuals (seedlings, juveniles and reproductive) over two 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, but also 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 it will change in subsequent years.

Keywords: Atlantic Rainforest, fine-scale spatial genetic structure, genetic diversity, neighborhood size, seed collection
**Introduction**

Demographic and genetic studies are basic and powerful tools to understand 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 individuals that composes it, and population structure is susceptible to environmental conditions and dynamic processes, such as recruitment, mortality and growth rates, as well as intra- and interspecific 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 non-sprouting 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 (Saiter and Thomaz 2014) and 30°15’ S
(Carvalho 1994). This species was considered the most abundant and dominant tree in the
upper stratum of 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 AR (Ribeiro et
al. 2009, Vibrans et al. 2012), led to the placement of this species on the Brazilian List of
Endangered Plant Species (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 (diameter
at breast height – dbh > 10 cm) in remnants of 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 (dbh > 12.7 cm) (Veloso and Klein 1959) up to
20 to 50 reproductive individuals/ha (Reitz et al. 1978). Densities of 200 up to 600
individuals/ha 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*

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of this aggregation unknown, but also possible variations in the extent and the intensity of the spatial pattern between different categories of individuals (e.g., seedlings, juveniles or reproductive). 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 the 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) 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 Itajaí* (Serra do Itajai National Park: PNSI – 57,374 ha) and *Floresta Nacional de Ibirama* (Ibirama National Forest: FNI – 570 ha) (Figure 1). Vegetation in the study areas is classified as Ombrophilous Dense Forest (ODF), and the climate is described as subtropical humid (Cfa), according to Köeppen’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).

In order to collect demographic and genetic data, two permanent plots were established: a 15 ha plot (250 m x 600 m) in PNSI and a 15.5 ha plot (350 m x 430 m) in FNI. Two smaller plots subdivided into 10 m x 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 x 190 m) and 1.68 ha in FNI (120 m x 140 m) (Figure 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 diameter at breast height (dbh – 1.3 m); ii) juveniles, i.e., individuals with measurable dbh at 1.3 m up to 20 cm dbh; and iii) potentially reproductive (hereinafter, reproductive), i.e., individuals with dbh higher than 20 cm. We assessed seedling and juvenile individuals only in the smaller plots and the reproductive individuals in both smaller and bigger plots. We measured the height of seedlings and juveniles (up to 5 m) and the dbh of juveniles and reproductive individuals over two years, 2015 and 2016 in PNSI and 2016 and 2017 in FNI. Additionally, we evaluated individuals’ mortality, recruitment, density and increment (height and dbh). Spatial position of each individual was obtained by $xy$ coordinate system in each of the smaller plots and with GPS (Garmin GPSmap 76CSx) 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 confidence interval (95%) constructed through bootstrapping 1,000 times the density of each category of individuals in each subplot (10 m x 10 m). Confidence intervals (95%) were also constructed for the increment averages of height and dbh (bootstrap – 1,000 times). The increment values were obtained through two evaluations in each study site. The confidence intervals were constructed in R language (R Development 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 language (R Development Core Team 2015). Spatial independence between seedlings and reproductive individuals was tested using the bivariate extension of Ripley’s K $L_{12}(r)$ (Lotwick and Silverman 1982), as implemented in the “splancs” package (Rowlingson and Diggle 2015) in R language (R Development Core Team 2015). For both analyses, we used a radius ($r$) of 1 m to estimate $L(r)$ and $L_{12}(r)$, and the spatial pattern was evaluated up to half 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 bigger PNSI and FNI plots (reproductive), respectively. Deviations from Complete Spatial Randomness were tested through a confidence interval (95%) obtained from 1,000 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 bigger 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 seconds with an automatic homogenizer (6500 rotations per minute).

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 and 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 – Enzyme Commission 3.1.1.1), glucose-6-phosphate dehydrogenase (g6pdh, EC 1.1.1.49), glutamate dehydrogenase (gtdh, 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).

In order 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 ($\hat{A}$), average number of effective alleles per locus ($\hat{A}_E = 1/(1 - \hat{H}_E)$), observed ($\hat{H}_O$) and expected heterozygosity ($\hat{H}_E$) 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 1,000 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: $mva = N_{e\,(ref)}/(\hat{N}_e/n\cdot d)$, where $N_{e\,(ref)}$ is a reference value of 1,000 individuals, which was proposed by Lynch (1996) as sufficient to mitigate the effects of deleterious mutations, $n$ is the sample size, and $d$ 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 (PNSI = 2016 and FNI = 2017).

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) in order 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 confidence interval (95%) 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).

Neighborhood size ($\hat{N}_b$ – 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 $\hat{N}_b = -(1 - \theta_1)/b_k$ (Vekemans and Hardy 2004). In this formula, $\theta_1$ is the coancestry coefficient in the first class of distance (0 – 20 m), and $b_k$ is the regression slope of the coancestry coefficient value on the logarithm of spatial distance between individuals, but within plot distances, as
follows: 0 up to 560 m in PNSI and 0 up to 480 m in FNI. \( \bar{N}_b \) was estimated using
SPAGeDi, version 1.5 (Hardy and Vekemans 2002). Deme sizes (ha) were estimated for
each studied population based on \( \bar{N}_b \) values, as in the following equation: *deme size = \( \bar{N}_b / d \), 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
classes 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 (Figure 2).

Increment averages in height and dbh were similar between populations and
categories, except for seedlings from FNI, which showed statistically higher height
increment when compared to seedlings from PNSI (Table 2). All increment averages
presented high associated standard deviations (S), 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 (Figure 3). Seedling individuals from PNSI presented aggregation from 0 m up to 50 m in 2015 and 2016. Juveniles were aggregated from $\approx$5 up to 50 m in 2015 and 2016, and reproductive individuals presented aggregation from $\approx$5 up to 125 m (2015 and 2016) (Figure 3). In FNI, seedlings presented an aggregated spatial pattern from 0 m up to 60 m in 2016 and from $\approx$5 m up to $\approx$18 m in 2017. Juveniles presented no aggregation in 2016, but aggregation from 0 m up to $\approx$8 m was recorded in 2017. Reproductive individuals were aggregated from $\approx$2 m up to $\approx$75 m (2016 and 2017) (Figure 3). Aggregation intensity ($L(r)$) was higher for individuals from PNSI when compared to FNI (Figure 3). Seedlings and juveniles from PNSI and FNI presented spatial distribution independent of the spatial distribution of reproductive individuals (Figure 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 ($\hat{A}_e$) represented about 70% of $\hat{A}$ 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 deficit of heterozygotes (Table 3). Effective
population size was around 13% smaller than sample sizes, reflecting the \( \hat{f} \) estimates.

Minimum viable areas, taking a reference size of 1,000 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) (Figure 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 (Figure 5). All significant and positive coancestry values ranged from 0.037 (PNSI seedlings in 2015) up to 0.073 (FNI seedlings in 2016). Negative and significant \( \hat{\theta}_{xy} \) values were verified for reproductive individuals in the distance class of 140 m to 160 m and 180 m to 200 m in PNSI and FNI, respectively (Figure 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, correspondingly.

Discussion
This is the first attempt to understand demographic and genetic aspects of *O. catharinensis* over more than just one 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 the presented results and the following discussions are based on a two-year survey (genetic and demographic) in two populations. For that reasons, 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 (dbh> 10 cm) in remnants of 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 (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 up to 50 reproductive individuals/ha and from 200 up to 600 individuals/ha higher than 1 m. Such discrepancy between past (Veloso and Klein 1959, Reitz et al. 1978) and present estimates (Tarazi et al. 2010, Lingner et al. 2013, Table 1) 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% confidence interval = 95 years – 211 years).

The distribution of individuals per dbh classes (Figure 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 (Figure 2), owing to logging activities reported for PNSI until 1990 (MMA 2009). Therefore, theses 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

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 (Figure 3). In general, aggregation intensity (L(r)) was higher for individuals from PNSI, suggesting higher environmental heterogeneity in this study site when compared to FNI (Figure 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 hillsides (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 up to 60 m in 2016 and from ≈5 m up to ≈18 
m in 2017 (Figure 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 up to ≈8 m in 2017 (Figure 3).

Spatial distribution of seedlings was independent of the spatial distribution of 
reproductive individuals (Figure 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): 1) feeding from only one seed tree, dispersing seeds in suitable 
places for seedling recruitment (Moraes et al. 1999), which then favors aggregation and
spatial independence and 2) feeding from several seed trees, dispersing seeds along the area
(Moraes et al. 1999), and then favoring random distribution.

A comparison of PNSI and FNI based on differences in spatial distribution between
juveniles and reproductive individuals (Figure 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 ($\overline{H}_E$) for each population (PNSI = 0.222; FNI = 0.208) can also
be considered high when compared to average $\overline{H}_E$ 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, such as 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 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 can clarify this question.

Positive and significant fixation indexes ($\hat{f}$) estimates 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 1,000 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 1,000 individuals. Populations lose genetic diversity in 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 up to 40 m (Figure 5). These differences can be explained in several ways, i) methodological approaches: our reproductive category included only individuals with dbh > 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 (Figure 5). As discussed, spatial distribution of seedlings is independent of the spatial distribution of reproductive individuals for both populations (Figure 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 moschata*, 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 pattern of these categories in FNI (Figure 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 SGS level in the first distance class for seedlings (0 – 20 m) (Figure 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. Increase in SGS in older cohorts relative to younger cohorts can result from several factors, such as bottlenecks or founder effects, local adaptation owing to microhabitat selection, limited dispersal, and more overlapping generations in older cohorts compared to younger cohorts (Jones and Hubbell 2006).

Neighborhood size estimates ($N_b$) (PNSI = 44 individuals and FNI = 48 individuals) can be considered intermediate when compared to 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 in order 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* in order 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 in order to avoid collection of seeds from closely related individuals. In order 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 (Figure 5). In order 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 (Figure 5). Therefore, these are also ranges where 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 in loss of genetic diversity and population dynamism over generations, therefore 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 two-year period, highlighting the importance of such
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 in more than one year.
Acknowledgements

We thank the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) and the researchers of Núcleo de Pesquisas em Florestas Tropicais for their support with the fieldwork. We also thank the Laboratório de Fisiologia do Desenvolvimento e Genética Vegetal for providing the necessary infrastructure required for the genetic analysis performed in this work. This study was funded by the Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC) (grant numbers 11939/2009, 18868/2011-9 and TR2013-3558), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) to TM, APB, MBL, NCFC and JZS, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) to MSR (304724/2010-6), MPH (140386/2013-0) and RCR (130894/2015-0).
References


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Wright, S. 1931. Evolution in mendelian populations. *Genetics* 16(2): 97–159. Available from...
Table 1. Demographic estimates in two populations of *Ocotea catharinensis* Mez. from the Atlantic Rainforest, Santa Catarina, Brazil.

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PNSI</td>
<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>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td></td>
<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>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<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>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FNI</td>
<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>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<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>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<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>
<td>0</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 not found individuals. Confidence intervals (95%) for density averages are between parentheses.
Table 2. Increment averages in height and diameter at breast height (dbh) in two populations of *Ocotea catharinensis* Mez. from the Atlantic Rainforest, Santa Catarina, Brazil.

<table>
<thead>
<tr>
<th>parameter</th>
<th>category</th>
<th>increment</th>
<th>S</th>
<th>min</th>
<th>max</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNSI height (m)</td>
<td>seedlings</td>
<td>0.07 (0.05/0.08)</td>
<td>0.10</td>
<td>0</td>
<td>0.64</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>juveniles</td>
<td>0.14 (0.09/0.2)</td>
<td>0.17</td>
<td>0</td>
<td>0.68</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>juveniles</td>
<td>0.12 (0.08/0.18)</td>
<td>0.20</td>
<td>0</td>
<td>0.80</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>reproductive</td>
<td>0.8 (0.58/1.02)</td>
<td>0.48</td>
<td>0</td>
<td>1.60</td>
<td>16</td>
</tr>
<tr>
<td>FNI height (m)</td>
<td>seedlings</td>
<td>0.13 (0.09/0.15)</td>
<td>0.11</td>
<td>0</td>
<td>0.55</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>juveniles</td>
<td>0.13 (0.07/0.19)</td>
<td>0.16</td>
<td>0</td>
<td>0.70</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>juveniles</td>
<td>0.15 (0.09/0.2)</td>
<td>0.18</td>
<td>0</td>
<td>0.60</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>reproductive</td>
<td>1.04 (0.76/1.33)</td>
<td>1.00</td>
<td>0</td>
<td>3.80</td>
<td>51</td>
</tr>
</tbody>
</table>

**Note:** PNSI, Parque Nacional da Serra do Itajaí; FNI, Floresta Nacional de Ibirama; S, standard deviation; min, minimum; max, maximum; n, number of individuals. Confidence intervals (95%) for increment averages are between parentheses.
Table 3. Genetic estimates for two populations of *Ocotea catharinensis* Mez from the Atlantic Rainforest, Santa Catarina, Brazil.

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>$\tilde{N}_e$</th>
<th>$\tilde{P}$</th>
<th>$\tilde{k}$</th>
<th>$\tilde{A}$</th>
<th>$\tilde{A}_e$</th>
<th>$\tilde{H}_E$</th>
<th>$\tilde{H}_O$</th>
<th>$\hat{f}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PSNI</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 15/16</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>
</tr>
<tr>
<td><strong>FNI</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<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 16/17</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:** n, sample size; $\tilde{N}_e$, effective size; $\tilde{k}$, total number of alleles; $\tilde{P}$, percentage of polymorphic loci; $\tilde{A}$, average number of alleles per locus; $\tilde{A}_e$, average number of effective alleles per locus; $\tilde{H}_E$, genetic diversity; $\tilde{H}_O$, observed heterozygosity; $\hat{f}$, fixation index; *, p < 0.05.
Figure 1. Map showing both study sites and details of permanent plots.
Figure 2. Demographic structure in two populations of *Ocotea catharinensis* Mez. from the Atlantic Rainforest, Santa Catarina, Brazil. PNSI: Parque Nacional da Serra do Itajaí; FNI: Floresta Nacional de Ibirama.
Figure 3. Standardized Ripley’s function (L(r)) for seedling, juvenile and reproductive individuals plotted against radius distances (m) for Parque Nacional da Serra do Itajaí (PNSI 2015/2016) and Floresta Nacional de Ibirama (FNI 2016/2017). Continuous lines represent observed L(r), and dashed lines represent the upper and lower confidence envelope (95%) expected under Complete Spatial Randomness. n represents the number of individuals included in each graphic.
Figure 4. Standardized bivariate Ripley’s function ($L_{12}(r)$) for seedling vs. reproductive individuals plotted against radius distances (m) for Parque Nacional da Serra do Itajai (PNSI 2015/2016) and Floresta Nacional de Ibirama (FNI 2016/2017). Continuous lines represent observed $L_{12}(r)$, and dashed lines represent the upper and lower confidence envelope (95%) expected under Complete Spatial Independence. $n$ represents the number of seedling individuals/reproductive individuals included in each graphic.
Figure 5. Coancestry coefficient ($\hat{\theta}_{xy}$) plotted against distance classes (m) for seedling, juvenile and reproductive individuals from Parque Nacional da Serra do Itajaí (PNSI 2015/2016) and Floresta Nacional de Ibirama (FNI 2016/2017). Continuous lines represent observed $\hat{\theta}_{xy}$, and dashed lines represent the upper and lower confidence envelope (95%). n represents the number of individuals included in each graphic.
Map showing both study sites and details of permanent plots.

210x148mm (300 x 300 DPI)
Demographic structure in two populations of *Ocotea catharinensis* Mez. from the Atlantic Rainforest, Santa Catarina, Brazil. PNSI: Parque Nacional da Serra do Itajaí; FNI: Floresta Nacional de Ibirama.

73x29mm (300 x 300 DPI)
Standardized Ripley’s function (L(r)) for seedling, juvenile and reproductive individuals plotted against radius distances (m) for Parque Nacional da Serra do Itajaí (PNSI 2015/2016) and Floresta Nacional de Ibirama (FNI 2016/2017). Continuous lines represent observed L(r), and dashed lines represent the upper and lower confidence envelope (95%) expected under Complete Spatial Randomness. n represents the number of individuals included in each graphic.

149x123mm (300 x 300 DPI)
Standardized bivariate Ripley’s function ($L_{12}(r)$) for seedling vs. reproductive individuals plotted against radius distances (m) for Parque Nacional da Serra do Itajaí (PNSI 2015/2016) and Floresta Nacional de Ibirama (FNI 2016/2017). Continuous lines represent observed $L_{12}(r)$, and dashed lines represent the upper and lower confidence envelope (95%) expected under Complete Spatial Independence. $n$ represents the number of seedling individuals/reproductive individuals included in each graphic.

119x79mm (300 x 300 DPI)
Coancestry coefficient ($\theta_{xy}$) plotted against distance classes (m) for seedling, juvenile and reproductive individuals from Parque Nacional da Serra do Itajaí (PNSI 2015/2016) and Floresta Nacional de Ibirama (FNI 2016/2017). Continuous lines represent observed $\theta_{xy}$, and dashed lines represent the upper and lower confidence envelope (95%). $n$ represents the number of individuals included in each graphic.