



Original Article

Mating System and Effective Population Size of the Overexploited Neotropical Tree (*Myroxylon peruiferum* L.f.) and Their Impact on Seedling Production

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Abstract

The reproductive system of a tree species has substantial impact on genetic diversity and structure within and among natural populations. Such information, should be considered when planning tree planting for forest restoration. Here, we describe the mating system and genetic diversity of an overexploited Neotropical tree, *Myroxylon peruiferum* L.f. (Fabaceae) sampled from a forest remnant (10 seed trees and 200 seeds) and assess whether the effective population size of nursery-grown seedlings (148 seedlings) is sufficient to prevent inbreeding depression in reintroduced populations. Genetic analyses were performed based on 8 microsatellite loci. *M. peruiferum* presented a mixed mating system with evidence of biparental inbreeding ($\hat{t}_m - \hat{t}_s = 0.118$). We found low levels of genetic diversity for *M. peruiferum* species (allelic richness: 1.40 to 4.82; expected heterozygosity: 0.29 to 0.52). Based on $N_{e(v)}$ within progeny, we suggest a sample size of 47 seed trees to achieve an effective population size of 100. The effective population sizes for the nursery-grown seedlings were much smaller ($N_e = 27.54\text{--}34.86$) than that recommended for short term ($N_e \geq 100$) population conservation. Therefore, to obtain a reasonable genetic representation of native tree species and prevent problems associated with inbreeding depression, seedling production for restoration purposes may require a much larger sampling effort than is currently used, a problem that is further complicated by species with a mixed mating system. This study emphasizes the

need to integrate species reproductive biology into seedling production programs and connect conservation genetics with ecological restoration.

Subject area: Conservation genetics and biodiversity

Keywords: conservation genetics, forest nurseries, forest restoration, microsatellite, outcrossing rate, restoration plantations

Tropical forests host the majority of the world's terrestrial biodiversity and supply ecosystem goods and services to a large share of humanity, especially poor communities relying on natural resources to survive (Ghazoul 2015). However, tropical forests have been substantially affected by high levels of deforestation and chronic human-mediated disturbances, especially in developing countries (Sloan and Sayer 2015). In particular, large tree species have been one of the biological groups most affected by fragmentation (Haddad et al. 2015) and overexploitation (Asner et al. 2005; Oliveira et al. 2008), which have driven their decline worldwide (Lindenmayer and Laurance 2016).

Ecological restoration has recently emerged as a central strategy to mitigate the aforementioned environmental problems and support the expansion of tree species populations in human-modified tropical landscapes (Alexander et al. 2011; Melo et al. 2013a). Since many native trees with high conservation value may not spontaneously recolonize degraded sites (Reid et al. 2015; Shoo et al. 2016), active restoration and enrichment planting with seeds and nursery-grown seedlings have become common strategies to reintroduce a myriad of large tree species that are typical of old-growth tropical forests (Cole et al. 2011; Rodrigues et al. 2011; Bertacchi et al. 2016). In this context, the conservation value of tropical restored forests may heavily rely on the seedling stocks supplied by forest nurseries (Brançalion et al. 2012). However, to succeed in this task, forest nurseries must supply seedlings with a reasonable level of genetic diversity to support a long-term persistence of reintroduced populations in restored forests (Williams 2001). Despite the growing recognition of the importance of genetics in guiding restoration efforts (Mijangos et al. 2015) little is known about the capacity of forest nurseries to produce seedlings with adequate genetic diversity.

In addition to the negative consequences for population structure, overexploitation of tree species may also result in detrimental impacts on mating, gene flow, and genetic diversity (Aguilar et al. 2006, 2008; Arruda et al. 2015). The preservation of large trees populations depends mainly on conserving genetic variability, which in turn enhances trees' adaptive potential and the probability of long-term survival and reproductive success in a changing biosphere (Namkoong et al. 2002; Barrett and Schluter 2008; Wan et al. 2014). A species' reproductive system is one of the key factors influencing overall genetic diversity (Charlesworth and Wright 2001). Understanding the mating system of a species may also offer insights into population dynamics (Sebbenn 2006; Barrett 2010) and inform seedling production strategies to achieve the best outcomes for genetic conservation. The effective population size also has an important influence on the capacity of a population to maintain its genetic characteristics across generations, and it is essential in the analysis of a population's viability in the mid- and long-term (Palstra and Ruzzante 2008; Espeland and Rice 2010).

The goal of this study was to describe the mating system and effective population size in a conserved population of the overexploited Neotropical tree, *Myroxylon peruiferum* L.f. (Fabaceae), and determine if the genetic diversity of nursery-grown seedlings is sufficient to prevent inbreeding in populations reintroduced in forest

restoration sites. We address the following specific questions: (a) What is the mating system of the species? (b) How many seed trees are required for seed collection to achieve an effective population size that would potentially prevent inbreeding depression? (c) What is the level of genetic diversity and effective population size of nursery-grown seedlings produced for restoration projects?

Materials and Methods

Study Site and Species

The study area is located in the Atlantic Forest biome of Brazil, a global biodiversity hotspot that includes 20 000 plant species, of which 8 000 are endemic (Myers et al. 2000; Laurance 2009). Originally covering more than 130 million ha, the Atlantic Forest biome currently covers 19 676 120 ha or 15% of its original area (SOS Mata Atlântica/INPE 2015). The remaining forest cover is mainly found as small, isolated fragments embedded in human-modified landscapes (Ribeiro et al. 2009). Within the Atlantic Forest, we focused our study on the "Interior" biogeographical zone, the second most threatened of this biome, of which only 7% of the original forest cover remains (Ribeiro et al. 2009).

The study species is *M. peruiferum* L.f. (Fabaceae), commonly known as cabreúva or balsam, a late-successional tree widely distributed throughout the Atlantic Forest, which is pollinated by bees and birds, and has wind-dispersed seeds (Yamamoto 2001; Yamamoto et al. 2007). The wood is very dense (0.85–0.90 g/cm³) and resistant, making it suitable for construction and furniture manufacturing, and its aromatic compounds have also led to its use in cosmetics and barrels for beverage ageing (Lorenzi 1992; Figliolia et al. 2006; Catão et al. 2011). Furthermore, the species has phytotherapeutic potential as an antibacterial agent (Ohsaki et al. 1999; Gonçalves et al. 2005; Carvalho et al. 2008). *Myroxylon peruiferum* has experienced a significant reduction in population size caused largely by timber exploitation, which has compromised the genetic diversity of the species and increased its vulnerability to extinction (Mamede et al. 2007). This species is widely used in restoration programs throughout the Atlantic Forest that aim to recover high levels of plant taxonomic diversity in degraded areas, with a particular focus on threatened species (Brançalion et al. 2012). However, little is known about the genetic diversity of the species that are included in these programs.

Initially, we assessed the mating system, genetic diversity, fixation index, and effective population size of *M. peruiferum* in a natural forest remnant population found in the Caetetus Ecological Station (2170 ha), São Paulo State, southeastern Brazil. This protected area is one of the best-preserved remnants within the "Interior" biogeographical region of the Atlantic Forest (Figure 1).

We further selected 4 forest nurseries that supply seedlings to restoration projects to assess the genetic diversity, fixation index, and effective population size of nursery-grown seedlings: 1) Flora Tiête (N1)—a private nursery located in the municipality of Penápolis, SP, that collects *M. peruiferum* seeds from approximately 30 seed trees in the region; 2) Refloresta (N2)—a private nursery located in Capão Bonito, SP that uses *M. peruiferum* seeds bought

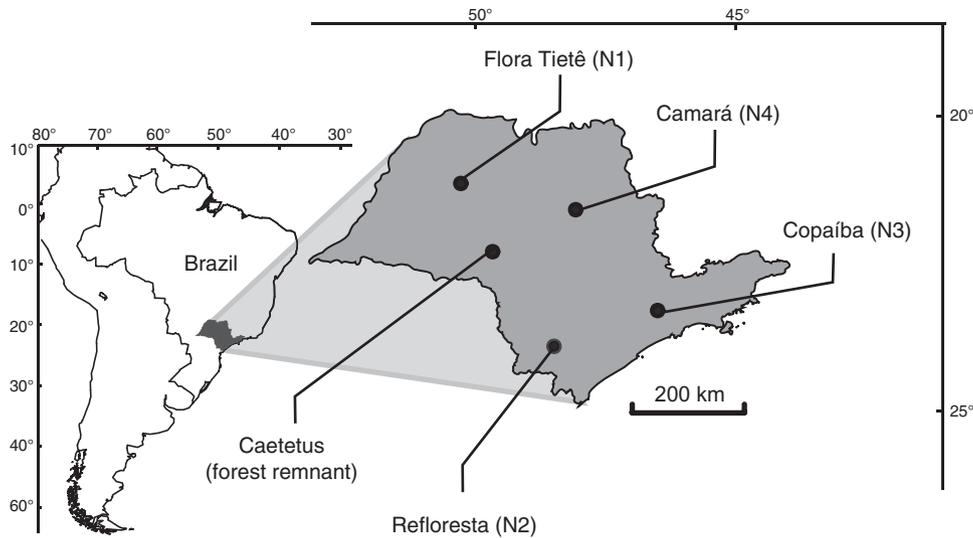


Figure 1. Map of the 5 study areas in São Paulo State, Brazil. Forest remnant, forest fragment reference population; N1, Flora Tiêete nursery; N2, Reffloresta nursery; N3, Copaíba nursery; N4, Camará nursery.

from a cooperative of seed collectors in the region; 3) Copaíba (N3)—a NGO forest nursery located in Socorro, SP, which collects *M. peruiferum* seeds from 3 seed trees; and 4) Camará (N4)—a private forest nursery in Ibaté, SP, that usually collects *M. peruiferum* seeds from 2 seed trees (Figure 1).

Sampling

To estimate the outcrossing rate of *M. peruiferum* we used 200 open pollinated progenies obtained from 10 seed trees in the aforementioned forest remnant. We collected seeds from all trees bearing seeds at the time of collection in November, 2012 (Figure 2). We also estimated genetic diversity, fixation index, and effective size for the following groups from the forest remnant: all 10 maternal genotypes, plus 2 groups of 5 and 2 randomly sampled seed trees; all 200 progenies arrays, plus samples of 100 and 40 progenies arrays; and 4 set of 37 randomly sampled seeds (hereafter pool1, pool2, pool3, and pool4). To estimate the genetic diversity, fixation index, and effective size of nursery-grown seedlings, we collected leaves from 37 randomly selected seedlings in each nursery, totaling 148 seedlings for the 4 nurseries. Samples were stored in liquid nitrogen until DNA extraction.

DNA Extraction and Genotyping

Genomic DNA was isolated following a protocol adapted from Doyle and Doyle (1990), in which we used an extraction buffer containing 2% CTAB, 20 mM EDTA, 100 mM Tris-HCl, pH 8.0, 2% PVP-40 (w/v), 1.42 M NaCl, and 3% betamercaptoethanol (v/v). Samples were amplified for 8 polymorphic microsatellite markers developed for *M. peruiferum* by Schwarcz et al. (2014): Mpe-C01, Mpe-C04, Mpe-C12, Mpe-E02, Mpe-E03, Mpe-F08, Mpe-G01, and Mpe-H10. Amplicons were separated using 5% polyacrylamide gel electrophoresis on a LI-COR 4300 DNA Analyzer (Li-Cor Biosciences, Lincoln, NE). Allele size was determined using the 50–350 bp IRDye700 and 800 ladder (Li-Cor) and SAGA v.3.3 software (Li-Cor).

Mating System Analysis

Based on the forest remnant population, we assessed the mating system using the mixed mating model (Ritland and Jain 1981) and the correlated mating model (Ritland 1989) implemented in

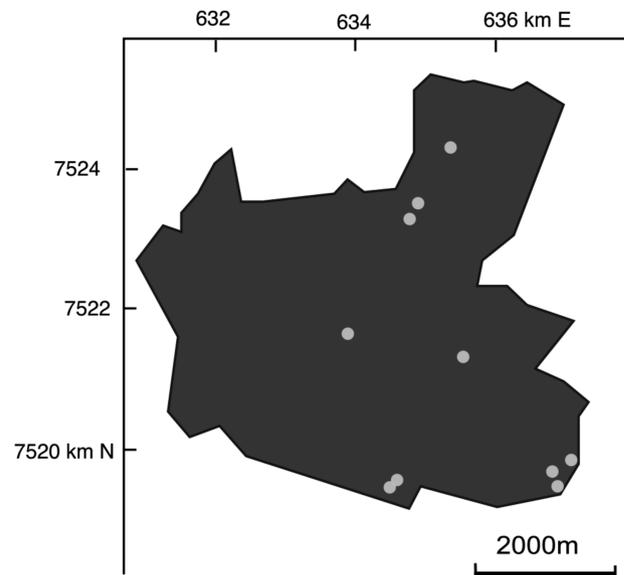


Figure 2. Map of the seed trees of *Myroxylon peruiferum* in the forest fragment found in the Caetetus Ecological Station in São Paulo State, Brazil.

the MLTR software (Ritland 2002). Numerical optimization using the population-level expectation maximization (EM) algorithm was used to obtain maximum likelihood estimate of parameters. We estimated the multilocus outcrossing rate (\hat{t}_m), single-locus outcrossing rate (\hat{t}_s), outcrossing rate among relatives ($\hat{t}_m - \hat{t}_s$) and multilocus paternity correlation (\hat{r}_p) using 5 seed-trees with known genotype and 5 seed-trees without genotypes. Thus, for the latter, the parameters were estimated indirectly. These parameters were used to estimate the effective number of pollen donors ($N_{ep} = 1/r_p$), and the average coancestry coefficient within the progeny: $\bar{\theta} = 0.125(1 + \hat{F}_p)[4\hat{s} + (\hat{t}_m^2 + \hat{s}\hat{t}_m\hat{r}_s)(1 + \hat{r}_p)]$, where F_p is the parental inbreeding coefficient (Ritland 1989), $s = 1 - t_m$ is the natural selfing rate, r_s is the correlation of selfing, and r_p is the multilocus paternity correlation. We also estimated effective number of pollen donors ($N_{ep} = 1/2\phi_{\hat{t}}$) and the average coancestry coefficient within progeny ($\bar{\theta} = 0.125(1 + \hat{F}_p)[4\hat{s} + (\hat{t}_m^2 + \hat{s}\hat{t}_m\hat{r}_s)(1 + 2\phi_{\hat{t}})]$) using the

coefficient of pollen pool structure ($\phi_{\hat{r}}$) in TwoGener (Smouse et al. 2001). The frequency of pairwise self-sibs (P_{SS}), half-sibs (P_{HS}), full-sibs (P_{FS}), and self-half-sibs (P_{SHS}) within progenies were estimated based on Sebbenn (2006).

The fixation index for the seed trees and progenies was calculated using the FSTAT software (Goudet 1995). The variance effective size ($N_{e(v)}$) within the progeny was estimated following Cockerham (1969), as adapted by Tambarussi et al. (2016). The number of seed trees necessary for seed collection to conserve or restore an effective population size of 100 that is recommended for short-term conservation to avoid inbreeding depression (Frankham et al. 2014), was estimated as $\hat{m} = N_{e(\text{reference})} / \hat{N}_{e(v)}$ (Sebbenn 2006). This estimate has 3 assumptions: 1) seed trees are not related; 2) each seed tree has a different set of pollen donors; and 3) selected seed trees do not mate with each other.

Analysis of Genetic Diversity

We assessed the genetic diversity of *M. peruiferum* species based on allelic richness (A_R), observed heterozygosity (H_O), and expected heterozygosity under Hardy–Weinberg equilibrium (H_E). To identify inbreeding, we used the fixation index (F_{IS}). These analyses were performed using R (R Core Team 2015) with the: diveRsite (Keenan et al. 2013) and PopGenKit (Paquette 2012) packages. The effective population size (N_e) was estimated following Cockerham (1969) with the coancestry coefficient inferred from the kinship coefficient using Nasson's formula (Loiselle et al. 1995), in the SPAGeDi 1.3 program (Hardy and Vekemans 2002). The confidence intervals were obtained from 1000 bootstrap replicates, resampling over loci.

Results

Mating System

For the forest remnant, the multilocus outcrossing rate (\hat{t}_m) was 0.740, the single-locus outcrossing rate (\hat{t}_s) was 0.622, and the selfing rate was 0.260 (Table 1). The rate of mating among

relatives ($\hat{t}_m - \hat{t}_s$) was 0.118, indicating biparental inbreeding and probable intra-population genetic structure. The multilocus paternity correlation (\hat{r}_p) was 0.233. The coefficient of pollen pool structure ($\phi_{\hat{r}}$) was 0.155. The number of pollen donors based on \hat{r}_p was 4.3 and on $\phi_{\hat{r}}$ was 3.2 (Table 1). The offspring from the forest population was mainly composed of half-sibs (42%), followed by self-half-sibs (38%), full-sibs (13%), and self-sibs (7%) (Table 1).

The coancestry coefficient within progeny was 0.217 and 0.223 and the variance effective size among offspring was 2.12 and 2.07 using \hat{r}_p and $\phi_{\hat{r}}$, respectively (Table 1). Based on this variance effective size, 47 seed trees are necessary for seed collection to conserve or restore an effective population size of 100 (Table 1).

Genetic Diversity

We found no significant differences for the estimates of genetic diversity (A_R and H_O) in nursery-grown seedlings (Table 2). The H_E estimate was significantly higher in the N4 than N3 nursery (95% confidence interval [CI]). Inbreeding was significantly higher in the N4 nursery than N2 and N3 (95% CI) and significantly different from zero. The N_e for the N1 and N4 nurseries was significantly lower than the sample size (Table 2).

Among maternal genotypes, the estimates of genetic diversity (A_R , H_O , H_E) and fixation index were not significantly different (Table 3), and inbreeding were not significantly different from zero. Furthermore, the N_e for maternal genotypes was not significantly different from the sample size (Table 3).

Among progeny arrays, the estimates of genetic diversity (A_R and H_O) and fixation index were not significantly different (Table 3). The H_E estimate for the progeny array of 200 was significantly higher than the progeny array of 40 (95% CI). The N_e for the progeny arrays were significantly lower than the sample size (Table 3).

For the 4 sets of randomly chosen seeds, the estimates of genetic diversity (A_R , H_O , H_E) and fixation index were not significantly different among thm. The N_e for each set of random seeds was significantly lower than the sample size (Table 3).

Discussion

Mating System

Mating system analysis showed a \hat{t}_m of 0.740, indicating a mixed mating system with a predominance of outcrossing and the likely presence of weak or no self-incompatibility mechanisms in the forest remnant. Species with a mixed mating system may have high levels of adaptability to environmental conditions in new areas and substantial evolutionary potential due to the occurrence of both selfing and allele recombination (Scariot et al. 1991). However, a mixed mating system can also lead to increased inbreeding depression in disturbed populations (Tambarussi et al. 2017). Because outcrossing rates can vary among populations, individuals, and reproductive events for a single plant (Sebbenn 2006; Feres et al. 2012; Karron et al. 2012), different populations may have different outcrossing rates. Therefore, the mating system of a species should be preferably determined based on many populations (Tambarussi et al. 2017). The use of a single population to estimate the mating system of *M. peruiferum* is thus a limitation of our study, which is justified by the lack of preserved populations of this rare, overexploited species available for seed sampling in the region.

The overall biparental outcrossing rate (0.118) for the forest remnant suggests existence of spatial genetic structure (SGS) due to mating among relatives and biparental inbreeding (Gaino et al. 2010;

Table 1. Estimates of the mating system parameters for the forest remnant population of *Myroxylon peruiferum*

Parameters	Ref (95% CI)
MLTR	
Number of mother-trees/number of seeds	10/200
Multilocus outcrossing rate (\hat{t}_m)	0.740 (0.646–0.807)
Single-locus outcrossing rate (\hat{t}_s)	0.622 (0.549–0.678)
Mating among relatives $\hat{t}_m - \hat{t}_s$	0.118 (0.060–0.174)
Multilocus paternity correlation \hat{r}_p	0.233 (0.106–0.335)
Percent of pairwise self-sibs (\hat{P}_{SS})	0.07 (0.04–0.13)
Percent of pairwise half-sibs (\hat{P}_{HS})	0.42 (0.32–0.53)
Percent of pairwise full-sibs (\hat{P}_{FS})	0.13 (0.05–0.19)
Percent of pairwise self-half-sibs (\hat{P}_{SHS})	0.38 (0.31–0.46)
Effective number of pollen donors (N_{ep})	4.3
Coancestry coefficient within progeny ($\hat{\theta}$)	0.217
Variance effective size ($N_{e(v)}$)	2.12
Number of seed trees (\hat{m})	47
TwoGener	
Coefficient of pollen pool structure ($\phi_{\hat{r}}$)	0.155
Effective number of pollen donors (N_{ep})	3.2
Coancestry coefficient within progeny ($\hat{\theta}$)	0.223
Variance effective size ($N_{e(v)}$)	2.23
Number of seed trees (\hat{m})	45

Table 2. Genetic diversity estimates for *Myroxylon peruiferum* in 4 commercial nurseries in São Paulo state

Nurseries	<i>n</i>	A_R (95% CI)	H_O (95% CI)	H_E (95% CI)	F_{IS} (95% CI)	N_e (95% CI)
N1	37	2.73 (2.12 to 3.00)	0.40 (0.33 to 0.47)	0.47 (0.43 to 0.49)	0.15 (−0.01 to 0.29)	31.54 (28.43 to 34.40)
N2	37	2.91 (2.25 to 3.25)	0.44 (0.39 to 0.50)	0.43 (0.38 to 0.47)	−0.03 (−0.15 to 0.09)	34.34 (31.39 to 37.83)
N3	37	3.01 (2.62 to 3.25)	0.42 (0.37 to 0.47)	0.43 (0.39 to 0.46)	0.04 (−0.08 to 0.16)	34.86 (32.39 to 39.29)
N4	37	2.92 (2.62 to 3.00)	0.35 (0.29 to 0.40)	0.51 (0.47 to 0.53)	0.34 (0.24 to 0.44)	27.54 (24.27 to 31.24)

n, number of sampled individuals; A_R , allelic richness; H_O , observed heterozygosity; H_E , expected heterozygosity under Hardy–Weinberg equilibrium; F_{IS} , fixation index; N_e , effective population size.

Table 3. Genetic diversity estimates for *Myroxylon peruiferum* seeds trees and progeny arrays from a forest remnant in São Paulo state

Groups	<i>n</i>	A_R (95% CI)	H_O (95% CI)	H_E (95% CI)	F_{IS} (95% CI)	N_e (95% CI)
10 seed trees	10	1.50 (0.30 to 2.25)	0.63 (0.45 to 0.81)	0.46 (0.40 to 0.56)	−0.33 (−0.66 to −0.17)	8.98 (7.80 to 10.06)
200 seeds	200	4.82 (4.13 to 5.38)	0.38 (0.36 to 0.40)	0.52 (0.50 to 0.55)	0.28 (0.22 to 0.32)	158.13 (149.62 to 165.46)
5 seed trees	5	1.53 (0.38 to 2.00)	0.60 (0.45 to 0.75)	0.43 (0.37 to 0.49)	−0.39 (−0.77 to −0.15)	5.24 (4.74 to 5.69)
100 seeds	100	4.52 (3.63 to 5.13)	0.35 (0.32 to 0.38)	0.50 (0.46 to 0.53)	0.30 (0.24 to 0.37)	76.95 (72.40 to 82.01)
2 seed trees	2	1.40 (0.50 to 1.75)	0.50 (0.14 to 0.86)	0.29 (0.11 to 0.46)	−0.80 (−1.31 to 0.41)	2.25 (1.88 to 2.50)
40 seeds	40	3.71 (2.88 to 4.25)	0.35 (0.30 to 0.40)	0.44 (0.39 to 0.49)	0.22 (0.11 to 0.31)	31.93 (29.85 to 33.81)
Simulations						
Pool1	37	3.18 (2.88 to 3.38)	0.37 (0.31 to 0.44)	0.43 (0.39 to 0.46)	0.15 (0.04 to 0.26)	32.88 (30.44 to 35.10)
Pool2	37	3.17 (2.88 to 3.38)	0.35 (0.30 to 0.41)	0.43 (0.39 to 0.46)	0.18 (0.07 to 0.30)	32.91 (30.23 to 36.01)
Pool3	37	3.21 (3.00 to 3.25)	0.39 (0.33 to 0.45)	0.47 (0.43 to 0.49)	0.17 (0.06 to 0.28)	31.75 (28.34 to 35.10)
Pool4	37	3.46 (3.13 to 3.75)	0.37 (0.32 to 0.43)	0.45 (0.41 to 0.48)	0.17 (0.07 to 0.27)	32.02 (29.88 to 34.14)

A_R , allelic richness; H_O , observed heterozygosity; H_E , expected heterozygosity under Hardy–Weinberg equilibrium; F_{IS} , fixation index; N_e , effective population size.

Forti et al. 2014). The dispersal mechanisms of a species can greatly influence SGS (Jordano et al. 2007; Hoban et al. 2014) and seed dispersal has a major impact on SGS in tropical species (Dick et al. 2008). *Myroxylon peruiferum* has large samara fruits dispersed by wind. Large samaras are often dispersed in clusters, with a consequent clustering of seedling recruitment (Greene and Johnson 1993) (Figure 2); this may explain the observed intrapopulation SGS found herein.

The effective number of pollen donors (N_{ep}) per offspring using the different parameters ($\phi_{it} = 3.2$ and $\hat{r}_p = 4.3$) suggests that few paternal parents contributed pollen to each offspring in the analyzed generation, indicating correlated mating. The correlated mating found in the *M. peruiferum* forest remnant population may be associated with the low population density of the species, at 2 individuals per hectare (Durigan et al. 2000). Low population density is linked to reduced levels of pollen diversification (Murawski and Hamrick 1991; Cascante et al. 2002), resulting in increased mating between a limited number of individuals.

The coancestry coefficient for each offspring using the 2 different parameters were higher ($\bar{\theta} = 0.217$ and 0.223) than expected for half-sib families ($\bar{\theta} = 0.125$), suggesting mixed parentage, and is consistent with the ratio of relationship types found among offspring (Table 1). The variance effective size within progeny using the 2 parameters was lower ($N_{e(v)} = 2.12$ and 2.07) than expected for a panmictic population ($N_{e(v)} \approx 4$), which has important implications for seed collection for breeding, conservation, and restoration programs (Sebbenn 2006).

The lower $N_{e(v)}$ within progeny is assumed to be related to inbreeding and biparental inbreeding found in this study, which is expected as we observed high levels of coancestry among seedlings

of progeny (Table 1). These processes can occur because of the limited number of seed trees sampled (10 seed trees), which included some isolated trees and some trees that are very close to each other (Figure 2). The limited sample size of seed trees and their spatial proximity may have influenced the results showing increased crossing among closely related individuals and reduced pollen diversity. Geographically isolated groups of trees or single isolated trees, as well as small or low-density populations, tend to show higher selfing rates (Dick et al. 2003; Lander et al. 2010; Moraes and Sebbenn 2011; Manoel et al. 2012; Tambarussi et al. 2017). Increased levels of pollen diversity can increase the genetic diversity and variance effective size within progeny (Picanço-Rodrigues et al. 2015), thus helping to maintain evolutionary potential (Sgrò et al. 2011).

Based on $N_{e(v)}$ within progeny from the forest remnant, for conservation and seed collection for restoration strategies, seeds from a large number of seed trees ($\hat{m} = 47$) should be collected to ensure a reliable effective population size for genetic conservation. Herein, we collected seeds from 10 seed trees, thus an additional 37 seed trees would need to be included in the sampling strategy of the present study. However, it is quite difficult to access *M. peruiferum* seed trees due to a combined consequence of its naturally low abundance in remnants, historical overlogging, and reduced cover of old-growth forests in the Atlantic Forest region. Macedo (1993) has noted the difficulty in collecting seeds from native species, which are commonly obtained from a few trees and in urban areas, leading to genetic problems that may affect the success of future planting efforts.

Other studies have also recommended seed collection from many seed trees to achieve the desired effective population sizes for genetic conservation. The BLM Seeds of Success program (USDI Bureau of Land Management 2012), for instance, suggests sampling seeds from

50 individuals if the mating system of the species or rates of selfing are unknown. Sebbenn (2002) also recommends collecting seeds from a larger number of seed trees for species that have some level of inbreeding.

Genetic Diversity

The values for genetic diversity parameters presented in our study are lower than those commonly observed in tropical tree species. This low genetic diversity can be attributed to low population density in association with the mating system of *M. peruiferum*. A reduction in the density of adult trees due to logging can lead to changes in genetic diversity patterns and the loss of alleles (André et al. 2008; Lacerda et al. 2008), further affecting seed harvesting for restoration purposes.

The excess of homozygosity found in progeny arrays (Table 3) compared to seed trees can be attributed to selfing, mating among relatives, and correlated mating. These forms of reproduction increase levels of inbreeding and relatedness found within offspring and decrease the heterozygosity within progeny (Tarazi et al. 2013). The inbreeding levels found herein are likely the result of individual isolation due to fragmentation. Population isolation can influence plant–pollinator interactions (e.g., reduce the number of pollinators) resulting in a negative impact on the reproductive system and gene flow, and increasing the levels of inbreeding (Lowe et al. 2005; Aguilar et al. 2006; Girão et al. 2007; Kettle et al. 2007; Aguilar et al. 2008; Lobo et al. 2013; Tambarussi et al. 2015).

The N_e in seed trees and nurseries (N2 and N3) were similar to the sample size, indicating that there is no inbreeding in these groups, as seen in Table 3. As the fixation indices for these samples were not different from zero, we expect no negative impacts on the genetic diversity in these groups. For the other groups, the N_e was lower than the sample size. This low N_e is potentially related to inbreeding, mating among relatives, and correlated mating, probably due to the mixed mating system of the species and fragmentation at the seed collection sites. A mixed mating system strongly affects the estimates of N_e (Alves et al. 2003). Recent human-mediated disturbances that reduce the number of reproductive individuals, such as harvesting and fragmentation, may have resulted in restricted gene flow and, consequently, contributed to reduced genetic diversity over generations (Johansson et al. 2007; De-Lucas et al. 2009; Shivaprakash et al. 2014).

Comparing the effective population size between nurseries and the groups of seeds with known mothers (pool1, pool2, pool3, and pool4), we observed that the N_e were similar among them and lower than that recommended for short-term ($N_e \geq 100$) and long-term ($N_e \geq 1000$) conservation of populations (Frankham et al. 2014). To reestablish a population with high levels of genetic diversity, thus enabling mid to long-term ecological viability, the reintroduced population must have an effective population size large enough to prevent inbreeding depression over time. One strategy to increase the N_e of *M. peruiferum* in nursery-grown populations is to collect seeds from a large number of seed trees; this could provide greater genetic diversity, which in turn enhances the viability of new populations. The contrasting numbers of seed trees that nursery N1 (30) and N4 (2) use for collecting *M. peruiferum* seeds highlight the challenges to obtaining an appropriate N_e in practice.

An alternative strategy to increase genetic diversity in seedling stocks in nurseries is the exchange of seeds and seedlings among nurseries (Brancaion et al. 2012). If we consider an initial population with seedlings from all 4 nurseries or all sets of random seeds,

we would have a sample with an effective population size of 129 and 125, respectively, thus falling within the recommended levels for short-term population viability (Frankham et al. 2014). However, the strategy of mixing genetic materials from distant forest nurseries or collection areas can increase the risk of outbreeding depression in new populations, which may result in a loss of fitness due to genetic dissimilarity (Edmands 2007). Although care should be taken with the origins of the seeds to be exchanged among nurseries, mixing seed lots across nurseries or collection areas within the same biogeographical region is a promising strategy to increase the genetic diversity of nursery-grown seedlings at reduced costs. In fact, the risks for inbreeding depression is expected to be much higher for this species than that of outbreeding depression.

Specific recommendations related to seed collection of Atlantic Forest endangered trees species are needed to support ongoing large-scale restoration programs like the Atlantic Forest Restoration Pact, a coalition of more than 270 organizations working to restore 15 million hectares of the biome by 2050 (Melo et al. 2013b). This study highlights the need for better integration of conservation genetics with restoration programs to take advantage of the growing global investment in ecosystem restoration (Menz et al. 2013). Integrating conservation genetics into restoration programs is paramount for the reestablishment of populations with the genetic potential to self-perpetuate in the context of global environmental change.

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Data Archiving

We have archived the primary data underlying these analyses as follows: Sampling locations and microsatellite genotypes: Dryad (doi: 10.5061/dryad.f7t62).

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