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Shelter from the storm: Restored populations of the neotropical tree *Myroxylon peruiferum* are as genetically diverse as those from conserved remnants

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ABSTRACT

One of the major strategies used to prevent extinction of forest species is restoration of previously depleted areas. In this paper, we investigate the ability of forest restoration to produce tree populations with high genetic diversity in previously deforested areas. We used nine SSR markers to genotype populations of two forest restorations and two areas of natural remnants. There were no significant differences between natural forest remnants and restored forests with respect to inbreeding levels ($f = 0.20$) or genetic diversity, as assessed by levels of heterozygosity ($H_S \sim 0.31–0.43$) and allelic richness (2.41–2.94). Instead, we found evidence of gene flow from neighbouring woods to restored forests. Although some populations may show a lower number of private alleles, this would be an expected result of a bottleneck effect in reduced populations such as those in forest restorations. Although the loss of these low frequency alleles has no major consequences for genetic diversity, the impact on population fitness in a scenario of environmental change is unpredictable.

1. Introduction

Deforestation remains one of the most important processes in environmental change (Lambin and Geist, 2006), with consequences ranging from removal and erosion of the genetic diversity of natural populations to extinction of plant and animal species.

Two major strategies have been used to prevent extinction of forests and their species: (1) protection and conservation of natural forest remnants and (2) restoration of native forests in deforested lands.

Whether the new population established in a previously deforested area will be viable in the long term is a critical issue for forest restoration ecology. Once the remaining natural populations have already shown their viability, those forest remnants are the best reference points concerning features to be observed in viable and healthy restored populations.

Aside from the ecological processes of recovery and reestablishment of connectivity between restored forests and nearby remnants, one should also be concerned with the genetic diversity in those recovering populations (Chazdon et al., 2017; Ribeiro da Silva et al., 2015). However, until recently, little attention has been paid to this aspect of reforestation. Nonetheless, many restoration ecology researchers have suggested that using a narrow genetic base, obtained from seeds of a small number of individuals, could cause founder effects (Hartl and Clark, 1997; Sebbenn, 2002; Sebbenn et al., 2003), with reduction of genetic diversity and increased inbreeding, thus making restored populations inviable in the long term.

On the assumption that the restored tree populations must have undergone founder effects and reproductive isolation, we can deduce as

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logical consequences that they may have low genetic diversity and structure as well as reduced gene flow with other forest areas. Thus, we asked ourselves the following three questions: (1) Do restored forest populations have lower intrapopulation genetic structure than natural ones? (2) Do restored populations suffer from lower genetic diversity? (3) Do restorations result in higher inbreeding rates than natural remnants?

Our goal was to perform a comparative study of microsatellite molecular markers (SSR) in natural and restored populations in order to evaluate similarities and differences between both types of populations and, therefore, to assess the likelihood of forest restorations reaching long-term viability.

We used nine nuclear microsatellite markers to genotype populations of tree species in two natural remnant forest populations and two forest restorations. These data were used to estimate genetic diversity and population structure.

2. Material and methods

2.1. Species

As a study model, we chose a tree species native to the South American Atlantic Forest commonly used in Brazil's forest restorations. This species was chosen due to its widespread distribution in the natural and restored forests we studied. *Myroxylon peruiferum* L.f. (Fabaceae – Leguminosae: Papilionoideae), commonly known as cabreuva, is found across the continent, especially in semideciduous forests. The species is a deciduous tree, heliophytic, with a mixed mating system (Silvestre et al., 2017), occurring both within the dense primary forest and in secondary formations. It is anemochoric, and its wind seed dispersion is assumed to occur at short distances due to the size and weight of seeds. Pollination was described as either ornithophilic or melittophilic (Lorenzi, 2002; Yamamoto, 2001; Yamamoto et al., 2007).

2.2. Study sites

The study was conducted in the Brazilian Atlantic Forest (BAF), the second major tropical forest in South America, holding high levels of biological diversity and endemism. The forest originally covered an area of 130,000 km^2 (15% of Brazil's territory), spreading through the majority of South America Atlantic coast and reaching the inner part of the continent (Collins, 1990; Hirota et al., 2003). Unorganized occupation has reduced its area to only 11.26% of the original, formed mostly by small isolated fragments (Ribeiro et al., 2009).

Leaf samples were collected from saplings and adults of *M. peruiferum* in four distinct areas of seasonal semideciduous forest in the Atlantic Forest complex (Table 1 and Fig. 1).

We selected two areas of natural remnants and two other restored forests aiming to compare differences in genetic diversity and structure, as well as inbreeding rates in both kinds of populations.

Our reference for a well-preserved continuous natural forest was the Caetetus Ecological Station (**Ref** Durigan et al., 2000; Tabanez, 2005), one of the last significant natural remaining Semideciduous Forests in the western highlands of São Paulo state, Brazil (IBGE, 1992).

Ref is a fairly extensive and well-preserved forest fragment containing many endangered tree species and representing the best possible example of a non-anthropic area in the region.

Santa Genebra (**Frag** for 'fragmented'), is one of the largest urban forests in Brazil, second only to the Tijuca Forest in Rio de Janeiro. Is a semideciduous forest (approximately 85% of its area). By its isolation from other forests, as well as its proximity to urban areas and historical human impacts, **Frag** provides a good example of a degraded natural forest.

Our first restoration area (**Rest1**) was planned to recover the surroundings of a municipal water reservoir in Iracemápolis, São Paulo. It was done from 1988 to 1990 in a range of 50 m from the reservoir banks, in formerly occupied sugarcane plantations (Rodrigues et al., 1992). The area is located next to patches of natural forest remnants. The seedlings used in this restoration were provided by two different nursery gardens. This area has a high diversity of species (140 species – mainly natives) and currently has a canopy approximately 20 m tall.

The second restoration area (**Rest2**) is in Cosmopolis municipality in São Paulo state, on a sugarcane company area and is named Usina Ester. The process of restoration of a riparian stretch of Usina Ester, cleared in 1900 and located in the hydrographic basin of the Jaguari River, began in 1955 to replace a drained pasture on the banks of the Jaguari River. This area is formed by a vegetation strip in which were planted 71 tree species (50 native and 21 exotic). The seedlings used in this restoration were obtained from the ESALQ park on the São Paulo University campus, which in turn is also an older forest restoration. All areas are located in a highly degraded landscape with low habitat cover (Ribeiro da Silva et al., 2015; IBGE, 1992).

We assumed that estimates made from data collected in natural remnants (**Ref** and **Frag**) represent characteristics of *M. peruiferum* populations that have experienced little or no disturbance by human activities and against which we could evaluate our results in restored populations.

2.3. Molecular markers and genotyping

We quantified the genetic diversity of *Myroxylon peruiferum* using nine previously developed specific microsatellite markers (Schwarcz and Bajay, 2014). Polymerase chain reactions (PCR) followed the previously described amplification conditions. We genotyped amplified fragments on a Li-Cor 4300 DNA Analysis System (Li-Cor Biosciences, Lincoln, NE, USA) and determined allele lengths using the 50–350 bp IRDyeR (Li-Cor) sizing standard and the Saga v.3.3 software (Li-Cor).

2.4. Data analysis

Microchecker software was used to verify the presence of null alleles. To test data adherence to expected frequencies in Hardy-Weinberg equilibrium, we performed exact tests considering an alternative hypothesis of heterozygote deficit using a Markov Chain algorithm or, when possible, the complete enumeration method. A G test was used to check for possible linkage disequilibrium a. For multiple tests, the probabilities were corrected via the sequential correction method with (alpha) equal to 0.05 (Rice, 1989). Both exact tests of adherence to Hardy-Weinberg and linkage disequilibrium were calculated using GENEPOP software (Raymond and Rousset, 1995).

To address the genetic structure of our populations, we examined the relationship between geographic and genetic distances using

Table 1
Study sites and their code, sample sizes, geographical coordinates and area.

Site code	Name	Samples	Latitude	Longitude	Area (Km^2)	City
Ref	Estação Ecológica de Caetetus	53	22°41′S	49°16′W	21,788	Gália
Frag	ARIE Mata de Santa Genebra	40	22°49′S	47°06′W	2518	Paulínia
Rest1	Cosmópolis restauration	46	22°39′S	47°12′W	0.250	Cosmópolis
Rest2	Iracemápolis restauration	29	22°35′S	47°31′W	0.500	Iracemápolis

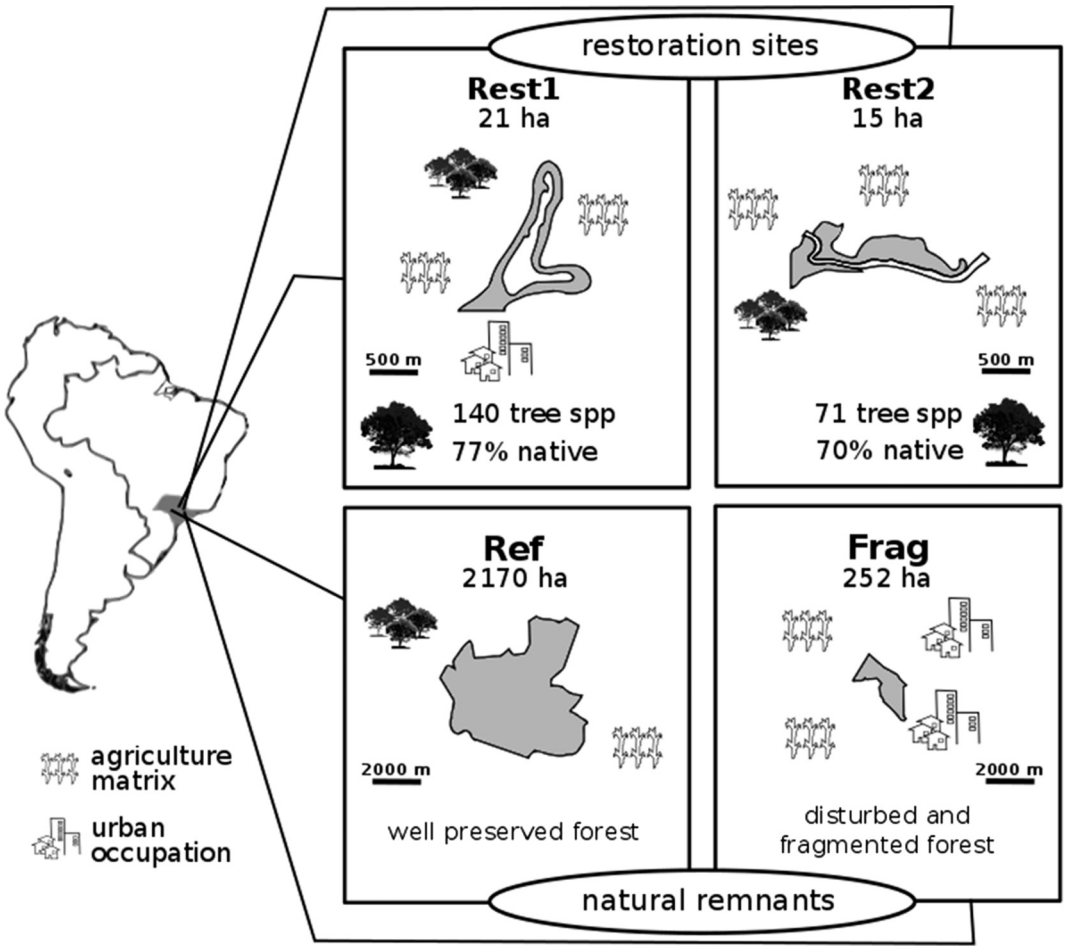

Fig. 1. Description of studied sites (grey) at the Atlantic Forest region of São Paulo state, Brazil. All sites are located in a matrix of agricultural business. Rest1 and Frag matrix also have urban occupation, while Ref, Rest1 and Rest2 are located near small natural forest fragments.

SPAGeDi software (Hardy and Vekemans, 2002). We used the kinship coefficient described by Loiselle et al. (1995), which has the advantage of not assuming Hardy-Weinberg equilibrium, thus not being influenced by the species mating system (Vekemans and Hardy, 2004). Average standard errors were obtained by jackknife resampling, and from this process, we determined confidence intervals at 95% probability for the average coancestry coefficient for each distance class. Lack of spatial genetic structure was tested in each distance class, using 10,000 permutations (Hardy and Vekemans, 2002). The magnitude of the spatial genetic structure was calculated using the Sp parameter (Vekemans and Hardy, 2004). The null hypothesis tested was the absence of spatial structure. When the b_{log} value (slope of the coancestry coefficient regression curve) is equal to zero, the null hypothesis is accepted.

The same data were subjected to a discriminant analysis of principal components (DAPC (Jombart et al., 2010) to investigate in more detail the genetic structure in each study site. Multivariate analyses in DAPC make no assumptions about genetic or reproductive population models and thus may be more effective than other traditional methods in identifying clinal genetic variation and hierarchical structure.

To better determine the genetic clusters present in each site, we performed a clustering analysis implemented by Jombart et al. (2010) using the *find.clusters* function of the adegenet software. We tested the number of clusters (K) from one to 20, with a burn-in of 1000 iterations followed by 10^7 iterations.

After identifying local clusters, we performed the DAPC considering this new assignment of genetic groups. DAPC was performed retaining principal components sufficient to explain 80% of total variance. The results were plotted for the first two principal components.

Being an exploratory analysis, DAPC does not perform tests of population models. To check the relevance of cluster separation in each area, we calculated theta pairwise estimators between clusters (F_{ST} estimator Weir and Clark Cockerham, 1984).

Finally, we plotted the location of genotyped individuals on an aerial photographic image of each site, identifying each cluster by a different colour (Fig. 3). This allowed us to observe that genetic clusters tended to be organized spatially, confirming the significant Sp results found previously.

To avoid mixing data from different genetic populations and creating unwanted bias such as the Wahlund effect, estimations of genetic diversity and inbreeding rates were made taking into account the previously identified genetic clusters, hence regarded as distinct populations.

We used GENETIX software (Belkhir et al., 1996) to calculate inbreeding coefficients (f) and theta estimators of Weir and Clark Cockerham (1984). Expected (H_S) and observed (H_O) heterozygosity were assessed through PopGenKit package of R (Paquette, 2012). We used the function bootstrapHet, performing 1000 bootstrap resamples, to calculate confidence intervals of H_S and H_O.

Allelic richness and private allelic richness were estimated using the program ADZE (Szpiech et al., 2008) which uses a rarefaction method to compare allelic diversity unbiased to differences in sample size.

We also investigated differences in genetic diversity related to age in each study site. For this, we classified individuals into two age classes. Since the height and diameter of neotropical trees are highly influenced by a number of different factors, including incidence of light, water, and nutrient availability, our classification criteria were based on field

observations during the flowering season. Individuals with DBH (diameter at breast height) up to 3.5 cm were classified as juveniles, while trees over 8.0 cm DBH were considered adults. Individuals with DBH between 3.5 and 8.0 were considered of indeterminate age and excluded from this analysis.

3. Results

3.1. Genetic variability

There was no evidence of genotyping errors or null alleles (results not shown). The exact tests indicated that six of the nine loci (Mpe-C01, C04-Mpe, Mpe-E02, Mpe-F08, F10-Mpe, Mpe-G01) showed significant deviation (P-value < .05) from expected genotypic frequencies at Hardy-Weinberg equilibrium after Bonferroni correction in a multilocus analysis. No linkage disequilibrium was detected in our data after applying sequential correction (Rice, 1989), neither when considering all sites nor when each site was analysed individually.

The per locus average number of alleles over all populations was 5.89. The F estimator (Weir and Clark Cockerham, 1984) was 0.355, showing high inbreeding rates for these *M. peruiferum* populations. On the other hand, *f* and theta estimators were 0.253 and 0.137 respectively. These values indicate the existence of moderate to high genetic diversity both within and among each study site; with higher variation within each site than between sites.

3.2. Answering questions

Question 1: Do restored forest populations present lower intrapopulation genetic structure than natural ones?

Correlation tests between genetic and geographical distances revealed the existence of a slight spatial genetic structure (SGS) at **Ref**, **Frag,** and **Rest2**, while **Rest1** did not show such a pattern (P-value > .05, Table 2). We identified distinct genetic clusters within all three study sites, in which there was SGS in a total of eight separate genetic clusters **Ref** (3 clusters), **Frag** and **Rest2** (2 clusters in each site). In **Rest1**, the most likely number of clusters was 1, confirming the absence of SGS (Fig. 2).

The DAPC was performed separating individuals according to the 8 previously identified clusters. After PCA transformation there were a sufficient number of principal components retained to keep 80% of the genetic variation. The three first discriminant functions were used for the DAPC. The results of pairwise theta estimates (Weir and Clark Cockerham, 1984) ranged from low (0.06) to high (0.42) and indicated significant structure between pairs of clusters identified in the same study sites (values bolded in Table 3).

In **Ref**, Cluster 3 comprised all individuals collected at the inner forest, a well-preserved area of thick jungle without clearings, not including any individuals outside this area. On the other hand, clusters 1 and 2 were located closer to the forest border or near human-made trails.

Table 2
Spatial Genetic Structure (SGS) in *M. peruiferum*. P-values maked with * indicates statistically significant structure.

Population	$F_{ij}(1)$	b_{log}	Sp	p-value
Ref	0.2045	−0.0051 (0.0009)	0.0065	.0000*
Frag	0.2134	−0.0159 (0.0016)	0.0202	.0000*
Rest1	0.0654	−0.0020 (0.0014)	0.0021	.1875
Rest2	0.2017	−0.0062 (0.0013)	0.0077	.0013*

In **Frag**, cluster 4 was distributed throughout the forest, except at the east-central portion where we identified only individuals of Cluster 5.

In **Rest2**, Cluster 7 was restricted to the north bank of the Jaguari River, while Cluster 6 included individuals on both sides of the river. We note that for Cluster 6, all individuals present on the north bank were juveniles (DBH ≤ 3.5 cm), while those on the south bank were all adults.

Answer: So we can say that intralocal structure is not exclusive to natural populations, but can be found in restored forests also. Besides, the strength of this local structure in restored populations may in some cases be similar to those found in natural ones, as can be observed in Table 3.

Question 2: Do restored populations suffer from lower genetic diversity?

We found no differences in heterozygosity, allelic richness or inbreeding coefficient between natural remnants and forest restorations when comparing areas (considering each area as a separate population – data not shown).

Comparison of age groups of each study site also showed no difference in heterozygosity values, inbreeding coefficient (*f*) or allelic richness between juveniles and adults (data not shown). However, at forest restoration sites, juveniles had a higher private allelic richness than adults (data not shown). But this result could be spurious as with this population number the difference can be to chance.

3.3. Genetic variability by clusters

We found no differences in heterozygosity or allelic richness between restored and natural remnant clusters (except for a slight difference of H_S between cluster 5 and 6). Table 4 shows the analysis of genetic diversity for each identified cluster. Only Cluster 6 showed no deviation from Hardy-Weinberg equilibrium. On the other hand, we observed mixed results for private allelic richness (PAR).

The data show that cluster 4 from **Frag** had a higher PAR than any of the restored populations, while clusters 6 and 7 (**Rest2**) had PAR values significantly lower than all others. Clusters 1, 2, 3 (**Ref**), 5 (**Frag**) and 8 (**Rest1**) did not differ significantly among themselves (Fig. 4A). Thus, although there were differences between clusters in PAR values, this may not be related to the nature (natural or restored) of the area to which they belonged.

Answer: The restored populations we investigate do not suffer from lower genetic diversity when compared to natural ones.

Question 3: Do restorations have higher inbreeding rates than natural remnants?

We found no differences in inbreeding coefficient between natural remnants and forest restorations when comparing areas with or without age discrimination.

After genetic clusters were identified, the inbreeding coefficient (*f*) was significantly different from zero only for Clusters 3, 7 and 8, corresponding to the permanent parcel of **Ref**, and the north banks of **Rest2** and **Rest1**, respectively (Fig. 4B).

Answer: After identify genetic clusters we were able to find inbreeding rates significantly different between some clusters, but that could not be related to a distinction between natural and restored clusters or populations.

4. Discussion

Brazilian Atlantic Forest (BAF) is considered as a biodiversity hotspot but has undergone an intensive process of environmental degradation that left only about 11.26% of its original area. Forest restoration is one of the two main strategies to prevent extinction of BAF and its especies but there are still few works dedicated to address the long term viability of these restored populations. Studies on the genetic diversity of already established forest restorations can provide

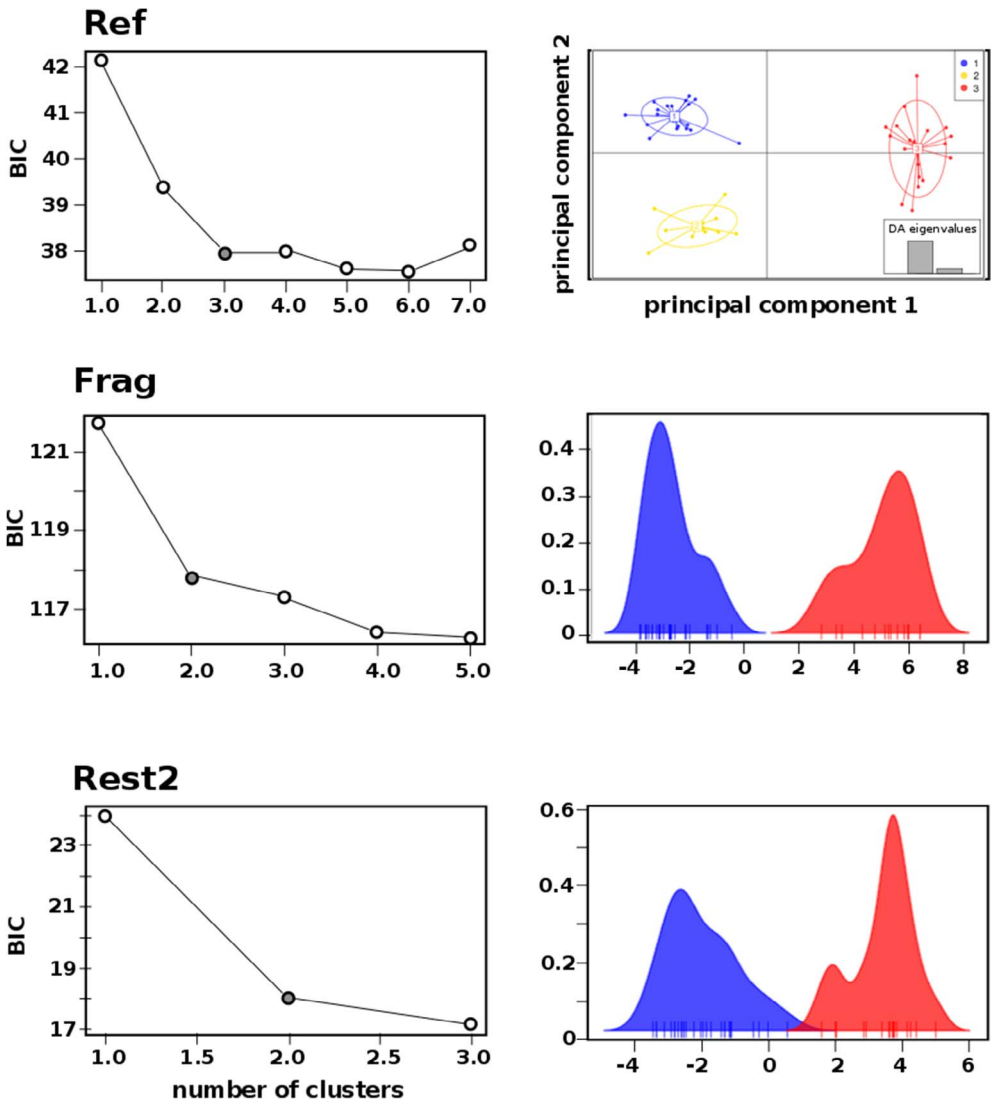

Fig. 2. Identification of genetic clusters in both forest remnants and in Restoration 2 (Rest2). For each area the first graphic shows decrease of baysian information content (BIC) by number of clusters, while second graphic shows discriminant functions (Loiselle et al., 1995).

important information to improve any flaw or reinforce good practices.

M. peruiferum is a species widely used in forest restoration projects and choosing it as our study species we aimed to shed some light on this issue. As we'll show below, our results did not found important differences between natural and restored populations with regards to genetic diversity. Thus the forest restorations we studied seems promising as self-sustaining *in situ* conservation areas for *M. peruiferum* genetic diversity.

The clustering analysis using principal components identified the genetic clusters previously suggested by significant SGS. Genetic structure was identified in both natural areas (Ref and Frag) and one of the restoration forests (**Rest2**). Thus, opposite to our preliminary hypotheses, it seems that inner genetic structure is not exclusive to natural remnants populations; instead, given due time, it can develop in restored populations, as well. We belive the genetic structure found in **Rest2**, in disagreement to ours expectations, could be due to its age (more than 60 years old as showed in methods), which we'll discuss below.

The largest number of clusters identified in **Ref** may be due to its much larger, continuous and better-preserved area. In **Rest1** the single cluster (8) identified agreed with the non-significant Sp obtained in SGS analysis. We believe that being a recent forest restoration, with adult individuals being mostly the originally planted trees, this population has had no time to develop inner genetic structure.

At **Rest2**, the long timespan since the restoration planting may have been responsible for allowing the development of a local genetic-spatial structuring. Another possible explanation could be the introduction of an *a priori* structuration at the moment of planting, by the grouped placement of genetically close seedlings. We dismissed this second explanation based on the discussion that follows.

At **Rest2**, Cluster 6 was present on both banks of the Jaguari river, while Cluster 7 was restricted to the northern bank of the restoration area. This result suggests the existence of gene flow between north and south banks of the river. That hypothesis is strengthened by the fact that all Cluster 6 individuals on the north bank were all juveniles, while those on the south bank were adults. Similarly, Sujii et al. (2017) also found evidence of gene flow between surrounding natural remnants and populations of *Centrolobium tomentosum* in these same restored forests. Therefore, the genetic structure observed by the presence of two distinct clusters in **Rest2** seems to be due to the introduction of new genotypes from the southern bank to the north bank of the river by gene flow.

No significant differences were observed between natural and restored forests with respect to genetic diversity, traditionally assessed by levels of heterozygosity and allelic richness.

The main differences between natural conserved remnants and forest restorations were the levels of private allelic richness (PAR), which ranked higher in one of the natural remnant clusters (4) and lower in Rest2 clusters (6 and 7). It is common for these private alleles, when present, to be present at low frequency, being less likely to be

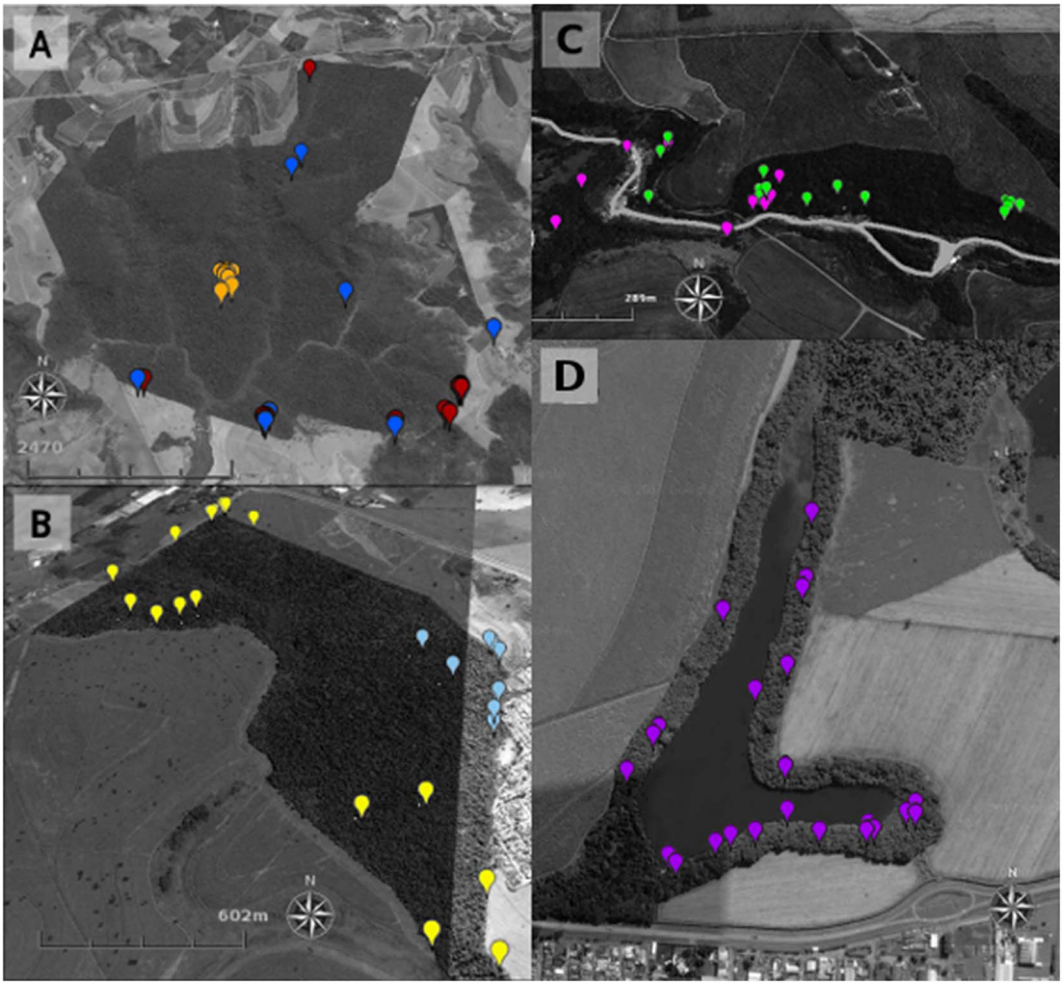

Fig. 3. Location of genetic clusters in each study site. Ref (A) with clusters 1, 2 and 3 (red, blue and orange), Frag (B) with clusters 4 and 5 (yellow and light blue), Rest2 (C) with clusters 6 and 7 (pink and green) and Rest1 (D) with cluster 8 (purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Genetic distances between pair of clusters according to theta estimator of Weir & Cockerham (Vekemans and Hardy, 2004). Bolded pairs of clusters in the same studying site.

Cluster	2	3	4	5	6	7	8
1	**0.0864**	**0.0635**	0.2980	0.1211	0.2835	0.0765	0.1224
2		**0.0809**	0.3033	0.1138	0.2835	0.1181	0.1529
3			0.2871	0.1340	0.2869	0.0947	0.1041
4				**0.2414**	0.4206	0.2369	0.2550
5					0.2209	0.0826	0.0749
6						**0.2032**	0.2763
7							0.0747

dispersed to neighbouring populations by gene flow due to simple random sampling effects (Slatkin, 1977, 1985; Allendorf, 1986; Ewens, 2012).

Our results are consistent with those of Carvalho et al. (2015) and Castilla et al. (2016) for other neotropical tree species. Although those studies focused only on natural areas, comparisons of different levels of forest cover found no differences in heterozygosity or allelic richness. Differences found in Castilla's work were related only to private allelic richness between types of forest cover.

In restored populations of *Centrolobium tomentosum*, Sujii et al. (2017) also found levels of genetic diversity, coancestry and inbreeding comparable to those of natural forest remnants.

Loss of rare alleles, with frequencies below 0.05, is a common occurrence when there is a population bottleneck due to drastic or prolonged reduction in population size (Allendorf, 1986). That loss of rare alleles, however, has little influence on heterozygosity since they contribute little to heterozygotes formation (Hartl and Clark, 1997).

Indeed, the implementation of a forest restoration may cause a strong founder effect, whose consequences are expected to be very similar to a bottleneck. This occurs because the seedlings are produced from seeds of a small sampling of adult trees.

In our case, for example, both restorations covered less than one square kilometre. That means the population size of each reinserted species will be small initially, and thus have a limitation of its maximum size. That is, there are two sampling processes: first, the location of a small number of seed provider trees, and second, the planting of a limited number of seedlings.

In older restorations, this problem can be even more serious since they were not designed with population genetic diversity in mind (Rodrigues et al., 2007). Only very recently have forest restoration projects begun to worry about using a broader genetic base (Rodrigues, 2009; Aerts and Honnay, 2011).

Rest2, begun in the 1950s, had no concern at all with genetic criteria. The seedlings were obtained from trees in the ESALQ park, which in turn is an older restoration. Thus, **Rest2** is the result of two consecutive sampling events, which would have produced founder effect and a narrow genetic base.

Rest1, in turn, was carried out with seedlings provided by two separate nursery gardens whose seeds were collected in different natural areas, in one of the first attempts to implant restorations with a broader genetic base. However, we do not have information on how many

Table 4
Genetic diversity (H_S – expected heterozigosity and H_O – observed heterozigosity) and inbreeding coefficient (*f*) estimated for *M. peruiferum*. Standard deviations and confidence intervals are shown in parenthesis.

	Cluster	N	Number of alleles	Allelic richness	Private allelic richness	H_S	H_O	f
Ref				2.769	0.122	0.376	0.407	0.1661
	1	12	22	(0.519)	(0.021)	(0.300, 0.397)	(0.333, 0.494)	(−0.0148, 0.3156)
				2.730	0.142	0.380	0.327	0.1052
	2	22	30	(0.448)	(0.038)	(0.303, 0.422)	(0.237, 0.427)	(−0.1440, 0.2323)
				2.941	0.184	0.380	0.365	0.2317
	3	19	28	(0.655)	(0.071)	(0.259, 0.402)	(0.259, 0.463)	(0.0729, 0.3449)
Frag				2.565	0.246	0.362	0.338	0.0833
	4	26	27	(0.438)	(0.058)	(0.316, 0.390)	(0.289, 0.388)	(−0.0504, 0.1708)
				2.736	0.122	0.429	0.386	0.2429
	5	14	26	(0.377)	(0.025)	(0.389, 0.488)	(0.282, 0.451)	(−0.0033, 0.3917)
Rest1				2.662	0.125	0.396	0.284	0.3524
	8	26	29	(0.352)	(0.038)	(0.332, 0.428)	(0.213, 0.342)	(0.1705–0.4767)
Rest2				2.411	0.048	0.309	0.360	−0.1019
	6	17	26	(0.363)	(0.019)	(0.264, 0.335)	(0.288, 0.424)	(−0.3290–0.0638)
				2.541	0.025	0.368	0.275	0.3436
	7	29	29	(0.372)	(0.007)	(0.300, 0.397)	(0.210, 0.351)	(0.2167, 0.4276)
Mean		20.6	28			0.396	0.3	0.200 (0.0911, 0.3536)

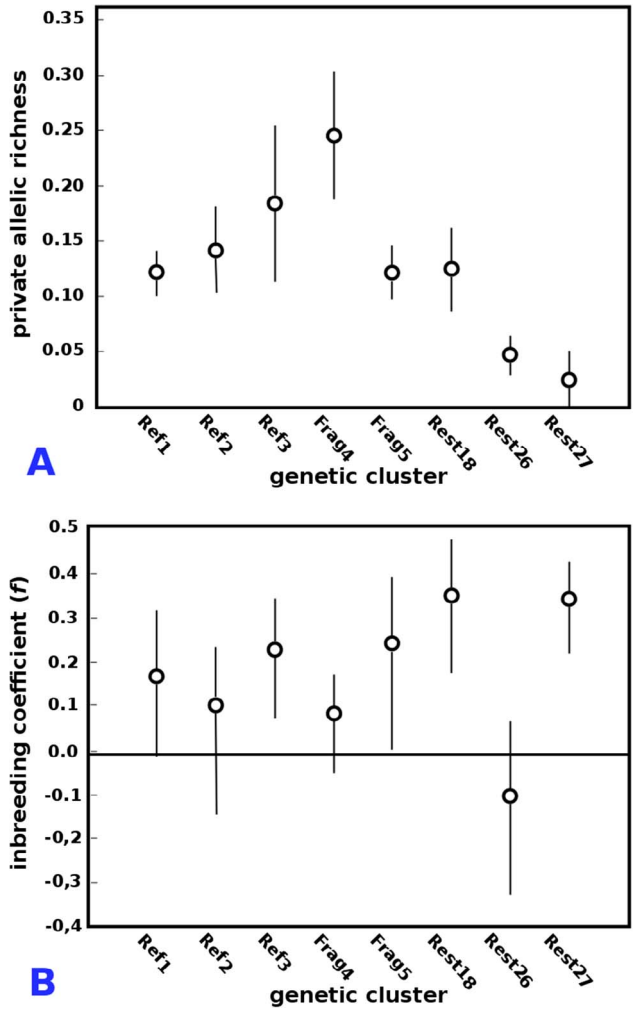

Fig. 4. (A) Private allelic richness (PAR) for each genetic cluster. Restored forest populations may show private allelic richness lower than those of natural remnants. That was the case for Rest2. (B) Inbreeding coefficient (*f*) for each genetic cluster. Only clusters 3, 7 and 8 have shown significant inbreeding.

mother trees this set of seeds represented; we assume it was a small number since *M. peruiferum* is an overly exploited species and thus is very difficult to find in the field.

Therefore, forest restorations in Iracemápolis (**Rest1**) and Cosmopolis (**Rest2**) would have suffered founder effects in their implementation. Although a strong effect has not been observed, as attested by genetic diversity as well as other data already presented, founder effect could still be an explanation for the loss of rare alleles and the difference observed in PAR (Slatkin, 1977, 1985; Allendorf, 1986; Ewens, 2012).

This may explain why the sites of forest restorations tend to have lower levels of private allelic richness compared to conserved remnants. We can, therefore, conclude that the results of heterozygosity and allelic diversity found in forest restoration do not differ from those we would expect in a bottleneck or founder effects occurring in small conserved remnants.

It is important to note that those higher levels of PAR found in juveniles of both forest restoration sites suggest the occurrence of gene flow from neighbouring forest patches, carrying new alleles to the forest restoration sites.

Ribeiro da Silva et al. (2015), founding higher number of species in Rest1 than in **Rest2**, suggested that this is likely to be due either to a higher number of species used during the restoration planting and nearby natural forests influencing its colonization rate.

The contribution of nearby forest remnants to species recolonization of restored areas by means of pollen and seed flow is indicated by Farah et al. (2017). For a genetic enrichment of restored populations by a similar process it would be necessary that species used in restorations to also be present in these surrounding natural remnants. Indeed, Allendorf and Luikart (2009) found a relevant intersection of plant species present in small remnant fragments and conservation units with bigger forest cover. When analysing the data for genetic cluster, we were able to identify differences in inbreeding coefficients, revealing significant *f* values for the forest restorations in contrast to non-significant values for conserved remnants. The exception is Cluster 3, which although belonging to a natural well-conserved site, presents significant *f*. We can consider that forest areas with high tree density population tend to hinder anemochory seed dispersal by reducing winds intensity, favouring greater inbreeding. Indeed, Howe and Smallwood (1982) indicates that in more open environments, seeds dispersed by wind may have higher chances of reaching long distances.

The small area and relative isolation of the other two clusters (7 and

8), located in forest restoration sites, should be the main causes for the significant inbreeding identified.

5. Conclusions and management implications

In general, we can say that in the *Myroxylon peruiferum* populations that we studied, there were no observed significant differences between natural forest remnants and restored forests.

To be able to persist through environmental changes, a population needs to maintain genetic variation in genomic regions related to adaptation. Those variable regions would then be selected by the new environmental conditions, due to better reproductive success of individuals with higher fitness (Allendorf and Luikart, 2009). Although genetic diversity in neutral regions of the genome does not guarantee adaptive potential, there is a correlation between neutral levels of genetic diversity and population fitness (Reed and Frankham, 2003). Thus, the levels of genetic diversity observed in our *M. peruiferum* restored populations, since they were similar to those of natural remnants, suggests a promising prognosis in regard to population long-term viability from the genetic point of view. This may be good news for forest restoration, since it seems to be an effective strategy for biodiversity conservation.

However, we must consider the fact that it is impossible to predict how important lost rare alleles will be in the future, with the prospect of continued climate change. Thus, although forest restoration can be a useful tool for environmental conservation, we must continue our efforts to preserve natural remnants.

Finally, we note that all studied sites, regardless of size, had some level of private alleles. In a study with adult subjects from four other tree species, differences in allelic richness between natural remnants and restored populations ranged from a slight reduction to a slight increase or no difference at all. This indicates that the challenge of restoring genetic diversity can vary among species. Nevertheless, in all cases the effect on the expected heterozygosity was also negligible when compared to natural remnants (Zucchi et al., 2017).

Farah et al. (2017), analysing data from 147 forest fragment sites, found that those fragments hold a number of species which exceeds that of species found in units of public conservation by 90%. This suggests that small forest remnants within an agricultural matrix have an important role in biodiversity conservation.

These data lead us to believe that even small forest fragments can host alleles unique to those sites. Our work leads us to recommend the conservation not only of large forest areas but also of small fragments, natural or restored, due both to their role as keepers of connectivity and gene flow, and to the unique alleles they preserve.

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References

Aerts, Raf, Honnay, Olivier, 2011. Forest restoration, biodiversity and ecosystem functioning. BMC Ecol. 11 (1), 29.
Allendorf, Fred W., 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biol. 5 (2), 181–190.
Allendorf, Fred W., Luikart, Gordon, 2009. Conservation and the Genetics of Populations. John Wiley & Sons.
Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F., 1996. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire génome, populations, interactions, CNRS UMR 5000, 1996–2004.
Carvalho, C.S., Ribeiro, M.C., Côrtes, M.C., Galetti, M., Collevatti, R.G., 2015. Contemporary and historic factors influence differently genetic differentiation and diversity in a tropical palm. Heredity 115 (3), 216–224.
Castilla, Antonio R., Pope, Nathaniel, Jaffé, Rodolfo, Jha, Shalene, 2016. Elevation, not deforestation promotes genetic differentiation in a pioneer tropical tree. PloS one 11 (6), e0156694.
Chazdon, R.L., Brancalion, P.H., Lamb, D., Laestadius, L., Calmon, M., Kumar, C., 2017. A policy-driven knowledge agenda for global forest and landscape restoration. Conserv. Lett. 10 (1), 125–132.
Collins, Mark (Ed.), 1990. EDI Seminar Series (Banco Mundial). No. GTZ-709 IUCN, Gland (Suiza).
Durigan, Giselda, Franco, G.A.D.C., Saito, M., Baitello, J.B., 2000. Estrutura e diversidade do componente arbóreo da floresta na Estação Ecológica dos Caetetus, Gália, SP. Rev. Bras. Bot 23 (4), 371–383.
Ewens, W.J., 2012. Mathematical Population Genetics 1: Theoretical Introduction. Springer Science & Business Media.
Farah, F.T., de Lara Muylaert, R., Ribeiro, M.C., Ribeiro, J.W., Mangueira, J.R.D.S.A., Souza, V.C., Rodrigues, R.R., 2017. Integrating plant richness in forest patches can rescue overall biodiversity in human-modified landscapes. For. Ecol. Manage. 397, 78–88.
Hardy, Olivier J., Vekemans, Xavier, 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol. Ecol. Notes 2 (4), 618–620.
Hartl, Daniel L., Clark, Andrew G., 1997. Principles of population genetics, third ed. Sinauer Associates, Sunderland, pp. 87.
Hirota, M.M., 2003. Monitoring the brazilian atlantic forest cover. In: Galindo-Leal, C., Câmara, I.G. (Eds.), The Atlantic Forest of South America: Biodiversity Status, Trends, and Outlook. Center for Applied Biodiversity Science e Island Press, Washington, D.C., pp. 60–65.
Howe, Henry F., Smallwood, Judith, 1982. Ecology of seed dispersal. Annu. Rev. Ecol. Syst. 13, 201–228.
IBGE, F. Manual Técnico da Vegetação Brazileira (Manuais Técnicos de Geociências n o 01). Rio de Janeiro, Brazil, 1992.
Jombart, Thibaut, Devillard, Sébastien, Balloux, François, 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 11 (1), 94.
Lambin, E.F., Geist, H.J. (Eds.), 2006. Land-Use and Land-Cover Change. Local Processes and Global Impacts. Global Change-The IGBP Ser. Springer-Verlag, Berlin.
Loiselle, Bette A., Sork, Victoria L., Nason, John, Graham, Catherine, 1995. Spatial genetic structure of a tropical understory shrub, Psychotria officinalis (Rubiaceae). Am. J. Bot. 1420–1425.
Lorenzi, Harri, 2002. Árvores Brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brazil. col. illus. Por Geog 4. Editora Plantarum, Nova Odessa, pp. 352.
Paquette, Sebastien Rioux, 2012. PopGenKit: Useful Functions for (batch) File Conversion and Data Resampling in Microsatellite Datasets. R Package Version, v. 1.
Raymond, Michel, Rousset, Francois, 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86 (3), 248–249.
Reed, David H., Frankham, Richard, 2003. Correlation between fitness and genetic diversity. Conserv. Biol. 17 (1), 230–237.
Ribeiro da Silva, F., Montoya, D., Furtado, R., Memmott, J., Pizo, M.A., Rodrigues, R.R., 2015. The restoration of tropical seed dispersal networks. Restor. Ecol. 23 (6), 852–860.
Ribeiro, M.C., Metzger, J.P., Martensen, A.C., Ponzoni, F.J., Hirota, M.M., 2009. The Brazilian Atlantic Forest: how much is left, and how is the remaining forest distributed? Implications for conservation. Biol. Conserv. 142 (6), 1141–1153.
Rice, William R., 1989. Analyzing tables of statistical tests. Evolution 43 (1), 223–225.
Rodrigues, Ricardo Ribeiro, Leitão Filho, Hermógenes de Freitas, Crestana, M.S.M., 1992. Revegetação do entorno da represa de abastecimento de água do município de Iracemápolis, SP. Simpósio sobre recuperação de áreas degradadas. Curitiba, PR. Anais. Curitiba, PR, sp.
Rodrigues, Ricardo R., Lima, Renato A.F., Gandolfi, Sergius, Nave, André G., 2009. On the restoration of high diversity forests: 30 years of experience in the Brazilian Atlantic Forest. Biol. Conserv. 142 (6), 1242–1251.
Rodrigues, Ricardo Ribeiro, Martins, Sebastiao Venancio, Gandolfi, Sergius, 2007. High Diversity Forest Restoration in Degraded Areas: Methods and Projects in Brazil. Nova Publishers.
Schwarcz, Kaiser Dias, Bajay, Miklos M., Macrini, Camila M.T., Salazar, Vera L.P., Souza, Anete P., Pinheiro, José B., Brancalion, Pedro H.S., Rodrigues, Ricardo R., Zucchi, Maria I., 2014. Microsatellite markers for the Cabreúva tree, *Myroxylon peruiferum* (Fabaceae), an endangered medicinal species from the Brazilian Atlantic Forest. Genet. Mol. Res. 13, 6920–6925.
Sebbenn, Alexandre Magno, 2002. Número de árvores matrizes e conceitos genéticos na coleta de sementes para reflorestamentos com espécies nativas. Revista do Instituto Florestal 14 (2), 115–132.
Sebbenn, Alexandre Magno, Kageyama, Paulo Yoshio, Vencovsky, Roland, 2003. Conservação genética *in situ* e número de matrizes para a coleta de sementes em população de Genipa americana L. Sci. Forest. 63, 13–22.
Silvestre, Ellida de Aguiar, Schwarcz, Kaiser Dias, Grando, Carolina, de Campos, Jaqueline Bueno, Sujii, Patricia Sanae, Tambarussi, Evandro Vagner, Macrini, Camila Menezes Trindade, Pinheiro, José Baldin, Brancalion, Pedro Henrique Santin, Zucchi, Maria Imaculada, 2017. Mating system and effective population size of the over-exploited Neotropical tree (*Myroxylon peruiferum* Lf) and their impact on seedling production. J. Hered. esx096. http://dx.doi.org/10.1093/jhered/esx096.
Slatkin, M., 1977. Gene flow and genetic drift in a species subject to frequent local extinctions. Theor. Popul. Biol. 12 (3), 253–262.
Slatkin, Montgomery, 1985. Rare alleles as indicators of gene flow. Evolution 39 (1),

53–65.
Sujii, P.S., Schwarcz, K.D., Grando, C., de Aguiar Silvestre, E., Mori, G.M., Brancalion, P.H., Zucchi, M.I., 2017. Recovery of genetic diversity levels of a Neotropical tree in Atlantic Forest restoration plantations. Biol. Conserv. 211, 110–116.
Szpiech, Zachary A., Jakobsson, Mattias, Rosenberg, Noah A., 2008. ADZE: a rarefaction approach for counting alleles private to combinations of populations. Bioinformatics 24 (21), 2498–2504.
Tabanez, Marlene Francisca, et al., 2005. Plano de manejo da Estação Ecológica dos Caetetus. Instituto Florestal Série Registros 29, 1–104.
Vekemans, Xavier, Hardy, Olivier J., 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. Mol. Ecol. 13 (4), 921–935.
Weir, Bruce S., Clark Cockerham, C., 1984. Estimating F-statistics for the analysis of population structure. Evolution 1358–1370.
Yamamoto, L.F., 2001. Florística e síndromes de polinização e dispersão em um fragmento de floresta estacional semidecídua montana, Município de Pedreira, Estado de São Paulo. Diss. Dissertação de Mestrado. Universidade de Campinas, Campinas, pp. 83.
Yamamoto, Leila Fumiyo, Kinoshita, Luiza Sumiko, Martins, Fernando Roberto, 2007. Síndromes de polinização e de dispersão em fragmentos da Floresta Estacional Semidecídua Montana, SP, Brazil. Acta Bot. Brazilica 21 (3), 553–573.
Zucchi, Maria I., Sujii, Patricia S., Mori, Gustavo, Viana, João Paulo, Grando, Carolina, Aguiar, Ellida, Schwarcz, Kaiser D., Macrini, Camila M.T., Bajay, Miklos M., Araújo, Fabiano, Siqueira, Marcos, Pereira, Alessandro, Souza, Anete, Pinheiro, José B., Rodrigues, Ricardo R., Brancalion, Pedro S., 2017. Genetic diversity of reintroduced tree populations in restoration plantations of the Brazilian Atlantic Forest. Restor. Ecol. http://dx.doi.org/10.1111/rec.12620/full.