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Genetic diversity of neotropical tree *Myrcia splendens* (Myrtaceae) in a fragment–corridor system in the Atlantic rainforest

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ABSTRACT

The presence of vegetation corridors in fragmented landscapes has high ecological importance to the gene flow of animals and plants. The landscape in the south of Minas Gerais State, Brazil is characterized by fragmented and disturbed ecosystems. However, there is some secondary vegetation corridors connected with remaining fragments of primary forest. In order to obtain information regarding of the importance of vegetation corridors in maintaining genetic diversity, we assessed the genetic diversity parameters of *Myrcia splendens* in a fragment–corridor system. Inter-simple sequence repeat markers (ISSRs) were used to assess the genetic diversity and genetic structure of *M. splendens*. The genetic structure was constructed using 168 trees distributed in five forest fragments and 104 trees distributed in four vegetation corridors. High genetic diversity of the species was indicated by Nei and Shannon indices in the fragments ($H_E = 0.37$, $I = 0.53$) and in the corridors ($H_E = 0.33$, $I = 0.48$). The historical gene flow was higher with neighboring corridors than with non-neighboring corridors. Differentiation among fragments was observed to be low with no correlation between genetic and geographic distances ($r = 0.057$, $P = 0.43$). Our results indicate that the fragment-corridor system in Minas Gerais State, Southeast Brazil, analyzed is of great importance for *M. splendens* in situ conservation plans.

1. Introduction

Tropical forest landscape changes due to expanding cattle-ranching and agricultural and silvicultural areas, in addition to inadequate exploration of wood resources, has led to the destruction and fragmentation of forest ecosystems. Besides the negative ecological effects, genetic variability and species fitness may decrease in a disturbed environment (Barret and Kohn, 1991; Heldrick and Miller, 1992; Telles et al., 2007; Young et al., 1996).

Gene dispersal for tree species inside fragmented landscapes faces several obstacles due to vector-dependent erosion of neutral and adaptive genetic diversity of populations (Couvet, 2002). Genetic consequences of forest fragmentation on plant species are more diverse than predicted by simple theoretical models (Bacles et al., 2004; Bacles and Jump, 2011; Kramer et al., 2008; Lowe et al., 2005) and the connection from one fragment to another through a vegetation corridor is essential for the maintenance of the genetic integrity of a species in these environments (Haddad, 2000; Mech and Hallett, 2001; Simberloff et al., 1992; Vieira and Carvalho, 2008).

In the landscape of the Alto Rio Grande region in the South of Minas Gerais State, forest fragments are connected by corridors of secondary vegetation – an important conservation system for maintenance of biological diversity. These areas are under high pressure, mainly due to urban expansion, logging and livestock. Assessment of genetic diversity of populations that occur in forest remnants and corridors of vegetation is crucial for developing efficient strategies for genetic conservation, and molecular markers can be used for this purpose. Inter-simple sequences repeats (ISSRs) markers are well-established in population genetics and have proven to be useful tools for detection of genetic diversity in tree and shrub natural populations and studies of cultivars (Li et al., 2010; Minsart et al., 2010; Oliveira et al., 2010; Viana e Sousa and Lovato, 2010). Studies on genetic aspects of conservation biology, such as the loss of genetic diversity in natural populations that are under human pressure, are truly addressed through detailed

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genetic studies of these populations, making use of molecular tools (Hamrick and Godt, 1996; Weising et al., 2005). There are few studies in Brazil on genetic diversity in natural populations of trees in fragments–corridors system (Vieira and Carvalho, 2008) and ISSR markers (Cota et al., 2011; Duarte et al., 2015; Lousada et al., 2011; Melo Júnior et al., 2015; Souza and Lovato, 2010).

As pointed out in Xiao et al. (2004) the use of ISSR markers has several advantages to assess genetic diversity in natural populations of trees. Analysis of genetic diversity and structure made from ISSR markers are more specific than the analysis with RAPD markers (Wolfe et al., 1998). The ISSR primers are based on SSR giving more region-specific annealing and, therefore, greater reproducibility of results, which is an advantage compared tothe analysis based on RAPD (Yang et al., 1996; Zietjiewicz et al., 1994). A lot of hypervariable loci found by ISSR markers makes them very interesting for studies in population genetics (Culley and Wolfe, 2001) and its final cost to the laboratory is low compared to other markers. However, the dominant nature of ISSR, and RAPDs markers, does not allow direct indication of those individuals which are homozygous or heterozygous.

In the present study, ISSR markers were used to analyze the genetic diversity of *Myrcia splendens* (Swartz) de Candolle on a fragment–corridor system. In this system, we expected to find low genetic differentiation between populations, since the vegetation corridors minimize the effects of genetic isolation caused by forest fragmentation. Our goal was to ask the following question: Are the fragment–corridor systems functional for maintenance of genetic diversity in *M. splendens* populations? This knowledge will provide baseline information for the adequate management and the conservation of small forest fragments and vegetation corridors in the regional landscape.

2. Materials and methods

2.1. Study species

M. splendens is a widely distributed tree species, naturally occurring from Mexico to Southern Brazil. The flowers of facultative outbreeding *M. splendens* are pollinated by bees, and the seeds are dispersed by small mammals, monkeys and birds (Torenzan-Silingardi and Oliveira, 2004). *M. splendens* occurs mainly at the edges of forest fragments and along the vegetation corridors. This species is highly abundant in the studied landscape, with a density about 122 ind. ha^1 in the forest fragments and 124 ind. ha^{-1} in the corridors.

2.2. Study site and populations

The populations studied are in Semideciduous Atlantic Forest fragments connected by vegetation corridors in Southeast Brazil, Minas Gerais State (Fig. 1). All reproductive individuals of *M. splendens* in five fragments (168 individuals) and four corridors (104 individuals) were sampled. These fragments have been subjected to different kinds of disturbance, with some types showing signs of trampling by cattle and timber harvests. The vegetation corridors were formed by colonization of trenches over the past 200 years. The matrix of fragments and corridors is primarily formed by pastures. Furthermore, the fauna present in these forest remnants uses vegetation corridors to explore other fragments, which is important for gene flow of *M. splendens* species and tree species dependent on these animals for seed dispersal.

2.3. DNA extraction and ISSR amplification

Genomic DNA was extracted from 10 mg of frozen leaves using a cetyltrimethyl ammonium bromide (CTAB) extraction method (Doyle and Doyle, 1987). Ten primers (Table 1) were selected from the twenty primers (synthesized by Biotechnology Laboratory of British Columbia University – Vancouver, British Columbia, Canada). Polymerase chain reaction (PCR) amplification was conducted in 12 μL consisting of 20 ng of template DNA, 10 X PCR buffer (500 mM Tris-HCl pH 8.0, 200 mM KCl, 0.25 mM BSA, 200 mM Tartrazine and 1% Ficoll), 2.6 mM $MgCl_2$, 0.25 mM of each dNTP, 0.125 U *Taq* DNA polymerase and 0.4 μM of ISSR primer. The PCR profile used to amplify the ISSRs was 94 °C for 3 min, 37 cycles of denaturation at 94 °C for 1 min, melting at 47 °C for 2 min, 72 °C for 2 min, and a final elongation step at 72 °C for 5 min. Amplified fragments were separated on 1.5% agarose gels stained with ethidium bromide in 0.5 × TBE (90 mM Tris, 92 mM boric acid, and 2.5 mM EDTA) buffer and photographed under ultraviolet light. Bands quantification was scored against 1000 bp DNA ladder standard.

2.4. Genetic analysis

The amplified DNA fragments were scored as presence or absence binary characters. The Polymorphism Information Content (PIC) was evaluated by using the following formula given by Bhat (2002): PIC = 2Pi (1-Pi) where Pi = frequency of different primers. The PIC indicates the ability of each marker to be found in two different states (presence/absence) in two plants taken randomly from the population. The PIC ranges from 0 to 0.5, indicating that the marker is monomorphic or present in 50% of the plants and absent in the other 50%, respectively (Roldan-Ruiz et al., 2000).

The resulting presence/absent data matrix of the ISSR genotypes was analyzed using POPGENE version 1.32 (Yeh et al., 1999) to estimate the following genetic indexes: the percentage of polymorphic loci, number of observed and effective allele, the expected heterozygosity (H_E) (Nei, 1973), diversity among populations (D_{ST}), the mean diversity within population (H_S), the total genetic heterozygosity (H_T), the Shannon index of diversity (I) (Shannon and Weaver, 1949; Lewontin, 1972), and the genetic differentiation among populations $G'_{ST} = G_{ST}(1 + H_S)/(1 - H_S)$, where $G_{ST} = D_{ST}/H_T$. Gene flow estimates (Nm) were calculated as $N_m = 0.5[(1 - G'_{ST})/G'_{ST}]$ (Hedrick, 2005; McDermott and McDonald, 1993).

Analysis of variance (one way ANOVA) was used to compare the magnitude of changes in levels of genetic diversity obtained by decomposing the total variance between samples and within populations. This analysis was complemented by *a priori* single Bonferroni test (Rice, 1989) to control false positive results, specifying what value of α is to be used in each test individually, adjusting the significance of the test for $P \leq \alpha$.

In order to test the correlation between genetic and geographical distances among populations, a Mantel test was performed with the randomization (Monte Carlo) method (1000 randomizations) in the PC-ORD 4.14 software (McCure and Mefford, 1997). Analysis of molecular variance (AMOVA) was used to determine the genetic structure within and among populations of *M. splendens* with the ARLEQUIN software (Excoffier et al., 2005). Significance level was detected via permutation test (n = 10,000). A Bayesian approach (Holsinger et al., 2002) was used to obtain genetic diversity (H_B), population differentiation ($G_{ST\text{-}B}$), gene flow ($Nm = 0.25(1 - G_{ST\text{-}B})/G_{ST\text{-}B}$), and the genetic divergence θ^B between pairs of populations with the HICKORY version 1.0.4 (Holsinger and Lewis, 2005). In this program, the value of θ^B can be calculated using four different models for dominant markers, specifically, assuming the model of free, ie, the program estimates the value of inbreeding; model $\theta^B = 0$ and $f = 0$, which set out θ^B and f values, respectively; and the complete model, which assumes all the previous possibilities. The simulations were obtained with Markov Chain Monte Carlo (MCMC) methods, which approximate *a posteriori* distribution of f and θ^B for any genetic data (Batistini et al., 2009). We

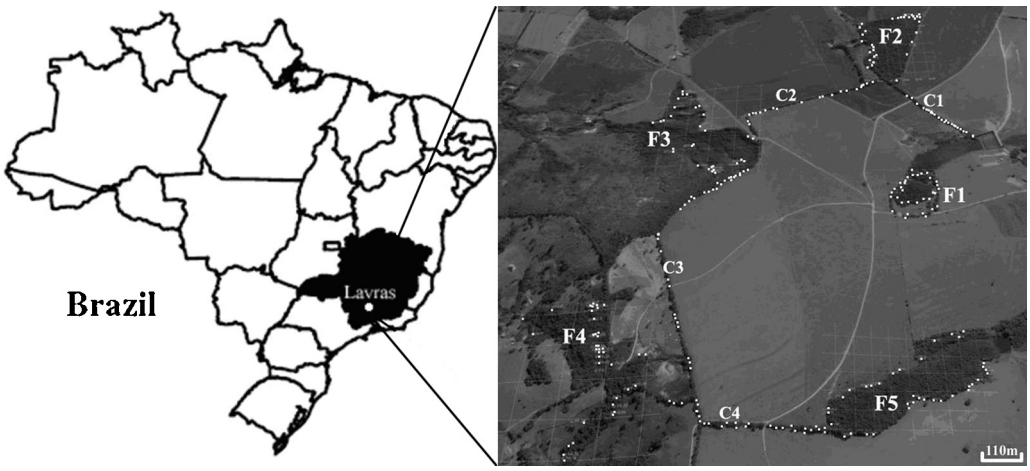

Fig. 1. Location of the studied fragment-corridor system with primary forest fragments (F1–F5) and secondary vegetation corridors (C1–C4) in Minas Gerais State, Southeast Brazil. The white points indicate the positions of the sampled individuals. Adapted from Brandão et al. (2011).

Table 1
Summary of data for the 10 ISSR primers used in the screening of *Myrcia splendens* (Swartz) de Candolle populations in Minas Gerais State, Southeast Brazil.

ISSR primers	Primer sequences (5'–3')	Number of loci	Number of polymorphic loci	Percentage of polymorphic loci (%)	Number of observed allele	Number of effective allele	PIC
AW3 (GT)6-RG	GTG TGT GTG TGT RG	7	7	100	2	1.8	0.464
BECKY (CA)7-YC	CAC ACA CAC ACA CAY C	7	7	100	2	1.7	0.401
CHRIS (CA)7-YG	CAC ACA CAC ACA CAY G	11	11	100	2	1.7	0.411
DAT (GA)7-RG	GAG AGA GAG AGA GAR G	3	3	100	2	1.5	0.342
GOOFY $(GT)_7$YG	GTG TGT GTG TGT GTY G	5	5	100	2	1.7	0.417
JOHN (AG)7-YC	AGA GAG AGA GAG AGY C	7	7	100	2	1.5	0.304
M1 CAA-(GA)5	CAA GAG AGA GAG A	7	7	100	2	1.8	0.441
MANNY (CAC)4-RC	CAC CAC CAC CAC RC	7	7	100	2	1.7	0.402
TERRY (GTG)4-RC	GTG GTG GTG GTG RC	10	10	100	2	1.6	0.348
UBC 843 (CT)8-RA	CTC TCT CTC TCT CTC TRA	6	6	100	2	1.6	0.376
Total		70	70	100	2	1.7	0.391

Note: R, purine (A or G); Y, pyrimidine (C or T). Number of polymorphic loci by 0.95 criterion, Nei, 1987. PIC: polymorphic information content.

chose the model that has the lowest value of Deviance Information Criterion (DIC). Spiegelhalter et al. (2002) proposed this method to choose between alternative statistical models, which takes into account both how a particular model fits the data as to how many parameters are required to do it.

A matrix of Nei's genetic distance was used to cluster the population by the unweighted pair group method with arithmetic averaging (UPGMA) using SAHN in the NTSYS program (Rohlf, 2000). Node consistency was evaluated by running 1000 bootstrap replicates using the TFPGA software (Miller, 1997).

In order to test the significant recent decreases in effective size due to recent colonization, the Bottleneck 1.2.02 program (Cornuet and Luikart, 1997) was used. This test is based on the principle that populations that have gone through a severe and recent genetic bottleneck show a faster reduction in the number of alleles than in H_E (Nei and Roychoudhury, 1974). The analysis was carried out on all loci assumed to fit an infinite allele model of mutation (IAM) (Kimura and Crow, 1964). The significance was assessed using the Wilcoxon signed rank test, based on 1000 replications

3. Results

3.1. Genetic diversity

The 10 selected primers generated 70 unambiguous and reproducible bands with sizes ranging from 350 to 3000 base pairs (bp). The number of bands varied from 3 to 11 with an average of 7 bands per locus (Table 1). The PIC values ranged from 0.305 to 0.464 with a mean of 0.391, thereby demonstrating the strong discriminatory power of the markers identified.

We detected high levels of genetic diversity in *M. splendens* on fragment–corridor systems (Table 2). The percentage of polymorphic loci was 83.2% in the corridors and 89.7% in the forest fragments. The total genetic heterozygosity (H_T) was 0.39 in the forest fragments and 0.37 in the corridors (Table 3). The average heterozygosity obtained with the HICKORY program was 0.33 and 0.35 in forest fragments and corridors, respectively. The average heterozygosity (H_S) of the forest fragments and corridors was 0.369 and 0.336, respectively. The variance analysis of genetic diversity between fragments and corridors did not present statistical differences (Table 2).

3.2. Genetic differentiation

According to the AMOVA analysis, the percentages of genetic variation within populations of *M. splendens* were 96.49% in the fragments and 91.15% in the corridors, thereby indicating that genetic differentiation was found mainly within fragments and corridors (Table 4).

Conversely, the population differentiation coefficient (G'_{ST}) showed a significant difference among individuals of *M. splendens* in fragments and corridors (Table 3). In the fragments and corridors, G'_{ST} was 0.117 and 0.185, respectively. These values demonstrate that genetic differentiation among and within populations in fragments contributed to 11.7% and 88.4% of the total heterozygosity, respectively. In the corridors, the percentages were 18.5% among and 81.5% within populations.

Table 2
Genetic diversity indices (mean ± standard error) for Myrcia splendens (Swartz) de Candolle populations in fragment (F)–corridor (C) system in Minas Gerais State, Southeast Brazil.

Populations	*A* (ha)	*L* (m)	*N*	H_E	H_B	*I*	$PP_{L\,(0.95)}$
F1	1.0	–	33	0.35 (±0.16)	0.308 (±0.026)	0.50 (±0.22)	87.14
F2	7.2	–	31	0.39 (±0.13)	0.338 (±0.021)	0.56 (±0.17)	94.29
F3	11.8	–	30	0.39 (±0.15)	0.347 (±0.021)	0.56 (±0.20)	91.43
F4	7.3	–	38	0.38 (±0.15)	0.323 (±0.026)	0.55 (±0.19)	91.43
F5	7.8	–	36	0.34 (±0.17)	0.303 (±0.025)	0.49 (±0.24)	84.29
C1	–	367	22	0.30 (±0.19)	0.320 (±0.028)	0.44 (±0.27)	75.71
C2	–	648	21	0.33 (±0.18)	0.333 (±0.026)	0.48 (±0.25)	81.43
C3	–	1039	36	0.36 (±0.14)	0.332 (±0.028)	0.53 (±0.19)	91.43
C4	–	542	25	0.34 (±0.18)	0.348 (±0.028)	0.50 (±0.24)	84.29
aa							
Average overall F	35.1	–	168	0.37 (±0.15)	0.324 (±0.023)	0.53 (±0.20)	89.71
Average overall C	–	2596	104	0.33 (±0.17)	0.333 (±0.025)	0.48 (±0.22)	83.21
aa							
F_{ANOVA}F				1.82 ^{ns}	–	1.76 ^{ns}	–
F_{ANOVA}C				1,28 ^{ns}	–	1.48 ^{ns}	–

(A) area of the fragments (ha) and length (m) of the vegetation corridors; population size (N), expected heterozygosity (HE), genetic diversity (HB) by Bayesian approach (Holsinger et al., 2002), Shannon index of diversity (I), and percentage of polymorphic loci PPL (0.95).

Table 3
Comparison of genetic estimates in *Myrcia splendens* (Swartz) de Candolle in the fragment-corridor system in Minas Gerais State, Southeast Brazil. H_T, total heterozygosity; H_{TB}, heterozygosity based on mean allele frequencies obtained with the HICKORY program; H_S, mean heterozygosity within populations; G'_{ST}, coefficient of gene differentiation; $G_{ST\text{-}B}$, Bayesian analog of Nei's G_{ST}; *Nm*' and Nm_B, gene flow estimates.

	H_T	H_{TB}	H_S	G'_{ST}	$G_{ST\text{-}B}$	θ^B	*Nm*'	Nm_B
Fragments	0.39 (±0.02)	0.333 (±0.023)	0.369 (±0.016)	0.117	0.027 (±0.004)	0.035 (±0.006)	3.76	6.9
Corridors	0.37 (±0.01)	0.353 (±0.025)	0.336 (±0.016)	0.185	0.057 (±0.007)	0.076 (±0.012)	2.2	3.04
F_{ANOVA}	0.87^{ns}	–	2.367^{ns}	9.722*	–	–	1.28^{ns}	–

* $P < 0.025$ complemented with *a priorit* Bonferroni (Rice, 1989) test; (mean ± standard error).

The full model was the most adequate to estimate genetic divergence (DIC-2788.28). $G_{ST\text{-}B}$ was higher in corridors (0.057) in comparison to fragments (0.027). However, these values obtained from the Bayesian approach were lower than those calculated by Nei's G'_{ST} method (Table 3). Likewise, the values of genetic differentiation (θ^B) were low for a set of populations. The genetic divergence supported by the Bayesian approach in the corridors was approximately double (0.076) that recorded in the fragments (0.035) (Table 3).

The historical gene flow (*Nm*) estimated by Nei's G'_{ST} was 3.76 individuals per generation in the fragments and 2.2 in the corridors. Using the Bayesian approach, the gene flow was determined to be 6.9 migrants in fragments and 3.0 in corridors. No significant differences among *Nm* for the set of fragment–corridor systems were detected (Table 5). In general, gene flow between fragments was greater than in corridors. The higher estimates of gene flow were observed between F1 and F5 ($Nm' = 11.04$ and $Nm_B = 31.00$) and the lower between F3 and F5 ($Nm' = 3.62$ and $Nm_B = 2.83$) forest fragments. The average gene flow was approximately twice as high in fragments ($Nm' = 7.00$ and $Nm_B = 10.41$) than in corridors ($Nm' = 3.50$ and $Nm_B = 3.17$). According to the variance analysis adjusted with the *t Bonferroni* test ($P < 0.001$, $F_{ANOVA} = 2.37$), *Nm* was higher between fragments F1 and F5 (11.04) than F1–F3 (4.15) and F4–F5 (7.35) (Table 5). The remaining values were not statistically different. Fig. 2 shows the proportion of contribution of gene flow among fragments.

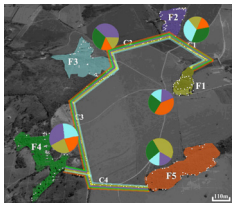

Fig. 2. Proportion of contribution of gene flow of system *Myrcia splendens* (Swartz) de Candolle in the regional landscape of a fragment–corridor in Minas Gerais State, Southeast Brazil.

The estimated Nei's genetic distance was 0.06 in the corridors and 0.033 in the fragments (Table 6). The smallest value of genetic distance in the corridors was 0.016 (C2–C3). These corridors connect fragments F2–F3 and F3–F4, respectively. The greatest genetic distance (0.106) was between corridors C1 and C4, which connect fragments F1–F2 and F4–F5, respectively. The highest genetic distance was between fragments F3 and F5 (0.061) and the smallest was between F1 and F5 (0.015).

The UPGMA data based on Nei's (1978) unbiased genetic distance matrix revealed a similarity of 92.5% to 98.5% among

Table 4
Analyses of molecular variance (AMOVA) for Myrcia splendens (Swartz) de Candolle in the fragment (F)-corridor (C) system in Minas Gerais State, Southeast Brazil, based on 70 ISSR markers.

Source of variation	d.f		Sum of squares		Variance components		(%) Total variance		*P*	
	F	C	F	C	F	C	F	C	F	C
Among populations	4	3	88.48	81.72	0.36	0.76	3.51	8.85	<0.0001	<0.0001
Within populations	163	100	1623.71	782.45	9.96	7.82	96.49	91.15	<0.0001	<0.0001
F_{ST} fragments	0.035									
F_{ST} corridors	0.088									
F_{ST} overall	0.075									

d.f. = Degree of freedom.

Table 5
Estimates of gene flow Nm' based on the Nei statistic (G_{ST}) and estimation of gene flow NmB based on the Bayesian statistic (colored in gray) between fragments (F) and fragments vs. corridors (C) in *Myrcia splendens* (Swartz) de Candolle in Minas Gerais State, Southeast Brazil.

	F1	F2	F3	F4	F5	C1	C2	C3	C4
F1	***	4.56	3.22	9.36	31	2.02	2.08	2.11	3.78
F2	5.07	***	16.41	13.63	4.37	2.55	3.42	2.62	9
F3	4.15*	9.43	***	9	2.83	1.57	2.15	1.96	4.37
F4	7.42	9.71	7.63	***	9.75	2.43	3.22	2.69	6.5
F5	11.04*	4.56	3.62	7.35*	***	2.17	2	1.9	4.85
C1	2.45	2.64	1.84	2.69	2.8	Average of *Nm*' in F			7
C2	2.86	3.69	2.6	3.65	2.9	Average of *Nm*' in FxC			3.5
C3	3.11	3.6	2.9	3.77	3.08	Average of Nm_B in F			10.41
C4	4.64	5.21	4.38	6.06	5.31	Average of Nm_B in FxC			3.17

* $P < 0.001$, $F_{ANOVA} = 2.37$, *F* test completed by *Bonferroni* test.

Table 6
Estimates of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) between corridors and fragments of *Myrcia splendens* (Swartz) de Candolle in Minas Gerais State, Southeast Brazil.

Populations	C1	C2	C3	C4	F1	F2	F3	F4	F5
C1	****	0.965	0.959	0.898	0.918	0.922	0.889	0.923	0.928
C2	0.035	****	0.983	0.917	0.928	0.944	0.919	0.943	0.929
C3	0.041	0.016	****	0.926	0.93	0.938	0.923	0.941	0.929
C4	0.106	0.086	0.076	****	0.957	0.961	0.953	0.967	0.962
F1	0.085	0.074	0.072	0.043	****	0.959	0.948	0.973	0.985
F2	0.08	0.056	0.063	0.038	0.041	****	0.981	0.981	0.954
F3	0.117	0.084	0.079	0.047	0.052	0.018	****	0.974	0.94
F4	0.079	0.058	0.06	0.033	0.026	0.018	0.026	****	0.973
F5	0.074	0.073	0.072	0.037	0.015	0.047	0.061	0.027	****

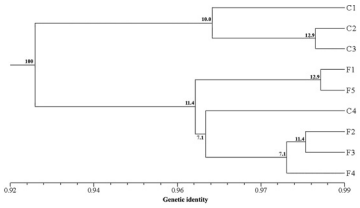

Fig. 3. UPGMA dendrogram of sampled *Myrcia splendens* (Swartz) de Candolle populations in the fragment–corridor system based on Nei's genetic identity. The numbers above the nodes indicate the consistency after 1,000 permutations.

populations (Fig. 3). Two clusters exhibit above 96% similarity, with the first being composed of three corridors (C1–C3), and the second being composed of five fragments (F1–F5) and corridor C4. Mantel tests provided no evidence for correlation between geographic distance and genetic structure ($r = 0.057$, $P = 0.436$). The bottleneck analysis procedures revealed a genetic bottleneck effect in the history of *M. splendens*, exhibiting excessive heterozygosity and a deficiency ratio of 65/5 ($P < 0.001$).

4. Discussion

4.1. Genetic diversity

The present study provides estimates of genetic diversity of the neotropical tree *M. splendens* in a fragment–corridor system. High levels of genetic diversity in the *M. splendens* populations were observed in fragments ($H_T = 0.39$) and corridors ($H_T = 0.37$). These values were higher than the overall value ($H_T = 0.22$) from a compilation of a set of ISSR-based studies of various angiosperm and gymnosperm taxa (Nybom, 2004).

In natural population studies, the percentage of polymorphic loci has been used as a measure of genetic diversity (Alexander et al., 2004; Ge and Sun, 2001; Xia et al., 2007). Fragments F1 (87.1%) and F5 (84.2%) were the fragments with the lowest proportion of polymorphic loci, with F1 being the smallest of the fragments (1.0 ha), and F5 being one of the most disturbed, mostly by cattle-raising. Indeed, these two fragments are the only ones receiving just one corridor (see Fig. 1).

Forest fragmentation of the studied area occurred approximately 200 years ago, and the effect of this process may not be reflected in the studied parameters because of the longevity of specie. However, the vegetation corridors in the landscape interconnecting the populations are of great importance for the maintenance of diversity. In this same corridor-fragment system, Vieira and Carvalho (2008) observed similar genetic diversity indices for the tree Protium spruceanum (Burseraceae), with diversity being higher in the fragments (average of 0.454) than in the corridors (average of 0.410). The authors suggested that the high number of reproductive individuals in the fragment allowed a high degree of recombination and multiple paternities, thereby favoring an increase in genetic diversity indices as functions of the increase in reproductive rate, as discussed in Franceschinelli and Bawa (2000) and van Treuren et al. (1993).

Genetic studies on populations of *M. splendens* not connected by corridors to check the dynamics on populations of specie, as well as studies of progenies, are important to determine whether genetic diversity is being maintained throughout generations. Indeed, studies of reproductive biology and phenology of *M. splendens* in this corridor-fragment system, in addition to genetic diversity parameters, will provide significant information for the conservation of this species.

4.2. AMOVA

We found that *M. splendens* populations across fragment–corridor system in Minas Gerais State, Southeast Brazil, were characterized by high genetic variation but low genetic differentiation (overall $F_{ST} = 0.075$). Estimates of genetic differentiation among outbreeding species obtained by dominant marker data and calculated through AMOVA are 28% on average (Nybom and Bartish, 2000). Vieira and Carvalho (2008) found similar levels of genetic diversity in *Protium* spruceanum (overall $F_{ST} = 0.028$) in this same fragment-corridor system. Our results are similar to those of other tropical outbreeding trees species

with long-life spans in which the major proportion of the genetic diversity is found within natural populations (Apte et al., 2006; Loveless and Hamrick, 1984; Nybom, 2004; White et al., 1999). As expected, *M. splendens* showed low genetic diversity among fragments. Genetic divergence among populations is reduced with the increase of gene flow via pollen and/or seeds.

4.3. Genetic differentiation

Gene exchange among fragments connected by vegetation corridors should be higher than those that are not. In this study, the *Nm* values were 3.7 in the fragments and 2.2 in the corridors. The gene flow values obtained by the Bayesian approach were higher than four migrants per generation among the fragments (6.9). Gene flow (*Nm*) higher than 4.0 migrants per generation is enough to counteract the effects of genetic drift (Slatkin, 1987; Wright, 1951). Furthermore, according to some authors (Mills and Allendorf, 1996; Wang, 2004) 1.0 to 10 migrants per generation among forest fragments can prevent the effects of mating of related individuals. When there is an effective gene flow among populations, homogenization of alleles occurs among them, and the population thus becomes panmictic (Hartl and Clark, 1997). Although fragments F1 and F5 are not connected directly by corridors, the results show that these fragments are not completely isolated. Besides, all fragments contributed significantly to gene flow, reinforcing the functionality of vegetation corridors for gene flow and, therefore, their importance in fragmented landscapes. In fact, no evidence of spatial genetic structuring in *M. splendens* has been observed in the primary formations (F1–F5) and in the vegetation corridors (C1 and C2), indicating that the genotypes are randomly distributed in this corridor-fragment system (Brandão et al., 2011). It is important to highlight that the gene flow estimated here reflects historic flow resulting in the genetic structure patterns observed to date. Other kinds of sampling, such as progeny and paternity analysis, can help us better understand contemporary gene flow in the studied landscape.

Above all, the high levels of *Nm* can be due to, among other factors, the species' large population size in the fragments and corridors and the efficiency of the dispersal of seeds and pollens. In fact, movement of wildlife can be seen in these corridors, illustrating the importance of fauna studies in this regional landscape. Besides, the levels of *Nm* in the species can be related with the fact that trees occur mainly at the edge of fragments and corridors (Fig. 1), which may favor the dispersion of seeds and pollens by animals (Smouse and Sork, 2004).

4.4. Genetic distance

In general, genetic distances were lower in interconnected fragments, except for corridor C1, which exhibits the smallest genetic distance to fragment F5 (0.074). Corridor C2 (interconnecting F2–F3) was least genetically distant to fragment F2 (0.056). Corridor C3 (connecting F3–F4) presented a low genetic distance to F4 (0.06). Corridor C4 had the smallest genetic distances with the fragments it interconnects, F4 (0.033) and F5 (0.037). Ours results indicate that the colonization of the ditches that originated the corridors possibly occurred from seed dispersion from the nearest fragments.

The results shown in Fig. 3 indicate a tendency of grouping according to the origin of populations (fragments and corridors). The vegetation corridors, possibly secondary forest formations, are grouped separately from the fragments, which are primary forest formations with the exception of corridor C4. This corridor presented high indices of gene flow among the five fragments (Table 5). The absence of correlation between geographical and genetic distances for *M. splendens* among fragments can be explained by the low genetic divergence among the fragments (Table 6) that exhibit high levels of gene flow.

4.5. Implications for conservation

Information on population genetics is an important tool for the conservation of forest fragments. These studies focus on the genetic conservation of species, as well as how the genetic diversity of populations could be affected by habitat fragmentation and loss of habitat (Jump and Penuelas, 2006; Lowe et al., 2005; Ortego et al., 2010; Young and Clarke, 2000). There is no clear information regarding the history of fragmentation in the studied area and the colonization of the ditches that gave rise to the vegetation corridors. Habitat fragmentation is relatively recent in the region and thus may have not yet affected the measured genetic diversity indices in the populations of *M. splendens*. However, the vegetation corridors interconnecting the remaining forest fragments are highly important in the landscape, due to their role in gene interchange among fragments, thereby minimizing the impacts of fragmentation. This genetic interchange among fragments can explain the low differentiation among the populations.

The vegetation corridors' role as a means for fauna movement, seed dispersion and gene interchange is a line of study that has been elucidated by many researching groups (Kirchner et al., 2003; Levery et al., 2005; Tewksbury et al., 2002; Vieira and Carvalho, 2008). As shown in this study, the conservation of these elements in the regional landscape is important for the conservation of *M. splendens* populations.

New studies describing the reproductive biology of *M. splendens* in association with genetic studies of other species with different pollination and dispersal systems should be undertaken to further knowledge of the dynamics of this regional landscape. Ecosystem management of these fragments is linked to the gathering of information concerning species with distinct ecological characteristics.

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References

Alexander, J.A., Liston, A., Popovich, S.J., 2004. Genetic diversity of the narrow endemic *Astragalus oniciformis* (Fabaceae). Am. J. Bot. 91, 2004–2012.

Apte, G.S., Bahulikar, R.A., Kulkarni, R.S., Lagu, M.D., Kulkarni, B.G., Suresh, H.S., Rao, P.S.N., Gupta, V.S., 2006. Genetic diversity analysis in *Gaultheria fragrantissima* Wall. (Ericaceae) from the two biodiversity hotspots in India using ISSR markers. Curr. Sci. 91, 1634–1640.

Bacles, C.F.E., Lowe, A.J., Ennos, R.A., 2004. Genetic effects of chronic habitat fragmentation on tree species: the case of *Sorbus aucuparia* in a deforested Scottish landscape. Mol. Ecol. 13, 573–584.

Bacles, C.F.E., Jump, A.S., 2011. Taking a tree's perspective on forest fragmentation genetics. Trends Plant Sci. 16, 13–18.

Barret, S.C.H., Kohn, J.R., 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk, D.A., Holsinger, K.E. (Eds.), Genetic and Conservation of Rare Plants. Oxford University Press, New York, pp. 3–30pp.

Batistini, A.P., Telles, M.P., Bertoni, B.W., Coppede, J.S., Môro, F.V., França, S.C., Pereira, A.M., 2009. Genetic diversity of natural populations of *Anemopaegma arvense* (Bignoniaceae) in the Cerrado of São Paulo State, Brazil. Genet. Mol. Res. 8, 52–63.

Brandão, M.M., Vieira, F.A., Carvalho, D., 2011. Fine-scale genetic structure of *Myrcia splendens* (Myrtaceae). Rev. Árvore 35, 957–964.

Cornuet, J.M., Luikart, G., 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144, 2001–2014.

Cota, L.G., Vieira, F.A., Melo Junior, A.F., Brandão, M.M., Santana, K.N.O., Guedes, M.L., Oliveira, D.A., 2011. Genetic diversity of *Annona crassiflora* (Annonaceae) in northern Minas Gerais State. Genet. Mol. Res. 10, 2172–2180.

Couvet, D., 2002. Deleterious effects of restricted gene flow in fragmented populations. Biodivers. Conserv. 16, 369–376.

Culley, T.M., Wolfe, A.D., 2001. Population genetic structure of the cleistogamous plant species *Viola pubescens* Aiton (Violaceae), as indicated by allozyme and ISSR molecular markers. Heredity 86, 545–556.
Doyle, J.J., Doyle, J.L., 1987. Isolation of plant DNA from fresh tissue. Focus 12, 13–15.
Duarte, J.F., de Carvalho, D., Vieira, F.A., 2015. Genetic conservation of *Ficus bonijesulapensis* R. M. Castro in a dry forest on limestone outcrops. Biochem. Syst. Ecol. 59, 54–62.
Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin ver 3.0: an integrated software package for population genetics data analysis. Evol. Bioinform. 1, 47–50, Online.
Franceschinelli, E.V., Bawa, K.S., 2000. The effect of ecological factors on the mating system of a South American shrub species (*Helicteres brevispira*). Heredity 84, 116–123.
Ge, X.J., Sun, M., 2001. Population genetic structure of *Ceriopstagal* (Rhizophoraceae) in Thailand and China. Wetlands Ecol. Manag. 9, 203–209.
Haddad, N., 2000. Corridor length and patch colonization by a butterfly, *Junonia coenia*. Conserv. Biol. 14, 738–745.
Hamrick, J.L., Godt, M.J.W., 1996. Conservation genetics of endemic plant species. In: Avise, J.C., Hamrick, J.L. (Eds.), Conservation Genetics: Case Histories From Nature. Chapman and Hall, New York, pp. 281–304.
Hartl, D.L., Clark, A.G., 1997. Principles of population genetics, Third ed. Sinauer Associates, Sunderland.
Hedrick, P.W., 2005. Standardized genetic differentiation measure. Evolution 59, 1633–1638.
Heldrick, P.W., Miller, P.S., 1992. Conservation genetics: techniques and fundamentals. Ecol. Appl. 2, 30–46.
Holsinger, K.E., Lewis, P.O., Dey, D.K., 2002. Bayesian approach to inferring population structure from dominant markers. Mol. Ecol. 11, 1157–1164.
Holsinger, K.E., Lewis, P.O., 2005. Hickory: A Package for Analysis of Population Genetic Data v.1.0.4. Department of Ecology and Evolutionary Biology. University of Connecticut, Storrs, CT.
Jump, A.S., Penuelas, J., 2006. Genetic effects of chronic habitat fragmentation in a wind-pollinated tree. Proc. Natl. Acad. Sci. U. S. A. 103, 8096–8100.
Kimura, M., Crow, J.F., 1964. The number of alleles that can be maintained in a finite population. Genetics 49, 725–738.
Kirchner, F., Ferdy, J.-B., Andalo, C., Colas, B., Moret, J., 2003. Role of corridors in plant dispersal: an example with the endangered *Ranunculus nodiflorus*. Conserv. Biol. 17, 401–410.
Kramer, A.T., Ison, J.L., Ashley, M.V., Howe, H.F., 2008. The paradox of fragmentation genetics. Conserv. Biol. 22, 878–885.
Levery, D.J., Bolker, B.M., Tewksbury, J.J., Sargent, S., Haddad, N.M., 2005. Effects of landscape corridors on seed dispersal by birds. Science 309, 146–148.
Lewontin, R.C., 1972. The apportionment of human diversity. Evol. Biol. 6, 381–398.
Li, S., Li, J., Yang, X.-L., Cheng, Z., Zhang, W.-J., 2010. Genetic diversity and differentiation of cultivated ginseng (*Panax ginseng* C. A. Meyer) populations in North-east China revealed by inter-simple sequence repeat (ISSR) markers. Genet. Resour. Crop Evol. 58, 815–824.
Loveless, M.D., Hamrick, J.L., 1984. Ecological determinants of genetic structure in plant populations. Annu. Rev. Ecol. Evol. Syst. 15, 65–95.
Lousada, J.M., Borba, E.L., Ribeiro, K.T., Ribeiro, L.C., Lovato, M.B., 2011. Genetic structure and variability of the endemic and vulnerable *Vellozia gigantea* (Velloziaceae) associated with the landscape in the Espinhaço Range, in southeastern Brazil: implications for conservation. Genetica 139, 431–440.
Lowe, A.J., Boshier, D., Ward, M., Bacles, C.F.E., Navarro, C., 2005. Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. Heredity 95, 255–273.
McCure, B., Mefford, M.J., 1997. PC-ORD: Multivariate Analysis of Ecological Data, Ver. 3.0. MjM Software, Gleneden Beach, OR.
McDermott, J.M., McDonald, B.A., 1993. Gene flow in plant pathosystems. Annu. Rev. Phytopathol. 31, 353–373.
Mech, S.G., Hallett, J.G., 2001. Evaluating the effectiveness of corridors: a genetic approach. Conserv. Biol. 15, 467–474.
Melo Júnior, A.F., de Carvalho, D., Brandão, M.M., Sousa, L.G., Vieira, F.A., Menezes, E.V., Royo, V.A., Oliveira, D.A., 2015. Spatial genetic structure of *Cavanillesia arborea* K. Schum. (Malvaceae) in seasonally dry Tropical forest: implications for conservation. Biochem. Syst. Ecol. 58, 114–119.
Miller, M.P., 1997. Tools for Population Genetic Analysis, Vers. 1.3. Flagstaff, Northern Arizona University.
Mills, L.S., Allendorf, F.W., 1996. The one-migrant-per-generation rule in conservation and management. Conserv. Biol. 10, 1509–1518.
Minsart, L.A., Zoro, Bi I.A., Dje, Y., Baudoin, J.P., Jacquemart, A.L., Bertin, P., 2010. Set up of simple sequence repeat markers and first investigation of the genetic diversity of West-African watermelon (*Citrullus lanatus* ssp. *vulgaris* Oleaginous type). Genet. Resour. Crop Evol. 58, 805–814.
Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 586–590.
Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University, New York.
Nei, M., Roychoudhury, A.K., 1974. Sampling variances of heterozygosity and genetic distance. Genetics 76, 379–390.
Nybom, H., Bartish, I., 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspect. Plant Ecol. Evol. Syst 3, 93–114.
Nybom, H., 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol. Ecol. 13, 1143–1155.
Oliveira, L.O., Venturini, B.A., Rossi, A.A.B., Hastenreiter, S.S., 2010. Clonal diversity and conservation genetics of the medical plant *Carapichea ipecacuanha* (Rubiaceae). Genet. Mol. Biol. 33, 86–93.
Ortego, J., Bonal, R., Munoz, A., 2010. Genetic consequences of habitat fragmentation in long-lived tree species: the case of the Mediterranean Holm Oak (*Quercus ilex*, L.). J. Hered. 101, 717–726.
Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.
Rohlf, F.J., 2000. NTSYS-pc. Numerical taxonomy and multivariate analysis system, Vers. 2.11. Applied Biostatistics, New York.
Roldan-Ruiz, I., Dendauw, J., Van Bockstaele, E., Depicker, A., De Loose, M., 2000. AFLP markers reveal high polymorphic rates in ryegrasses (Lolium spp.), Mol. Breed 6, 125–134.
Shannon, C.E., Weaver, W., 1949. The Mathematical Theory of Communication. Univ. of Illinois Press, Urbana.
Simberloff, D., Farr, J.A., Cox, J., Mehlman, D.W., 1992. Movement corridors: conservation bargains or poor investments? Conserv. Biol. 6, 493–504.
Slatkin, M., 1987. The average number of sites separating DNA sequences drawn from a subdivided population. Theor. Popul. Biol. 32, 42–49.
Smouse, P.E., Sork, V.L., 2004. Measuring pollen flow in forest trees: an exposition of alternative approaches. For. Ecol. Manage. 197, 21–38.
Souza, H.A.V., Lovato, M.B., 2010. Genetic diversity and structure of the critically endangered tree *Dimorphandra wilsonii* and of the widespread in the Brazilian Cerrado Dimorphandra mollis: implications for conservation. Biochem. Syst. Ecol. 38, 49–56.
Spiegelhalter, D.J., Best, N.G., Carlin, B.P., van der Linde, A., 2002. Bayesian measures of model complexity and fit. J. R. Stat. Soc. Ser. B 64, 483–689.
Telles, M.P.C., Diniz-Filho, J.A.F., Bastos, R.P., Soares, T.N., Guimarães, L.D., Lima, L.P., 2007. Landscape genetics of *Physalaemus cuvieri* in Brazilian Cerrado: correspondence between population structure and patterns of human occupation and habitat loss. Biol. Conserv. 139, 37–46.
Tewksbury, J.J., Levey, D.J., Haddad, N.M., Sargent, S., Orrock, J.L., Weldon, A., Danielson, B.J., Brinkerhoff, J., Damschen, E.I., Townsend, P., 2002. Corridors affect plants, animals, and their interactions in fragmented landscapes. Proc. Natl. Acad. Sci. U. S. A. 99, 12923–12926.
Torenzan-Silingardi, H.M., Oliveira, P.E.A.M., 2004. Phenology and reproductive ecology of *Myrcia rostrata* and *M. tomentosa* (Myrtaceae) in Central Brazil. Phyton 44, 23–43.
van Treuren, R., Bijlsma, R., Ouborg, N.J., van Delden, W., 1993. The effects of population size and plant density on outcrossing rates in locally endangered *Salvia pratensis*. Evolution 47, 1094–1104.
Viana e Sousa, H.A., Lovato, M.B., 2010. Genetic diversity and structure of the critically endangered tree *Dimorphandra wilsonii* and of the widespread in the Brazilian Cerrado Dimorphandra mollis: implications for conservation. Biochem. Syst. Ecol. 38, 49–56.
Vieira, F.A., Carvalho, D., 2008. Genetic structure of an insect-pollinated and bird-dispersed tropical tree in vegetation fragments and corridors: implications for conservation. Biodivers. Conserv. 17, 2305–2321.
Wang, J., 2004. Application of the one-migrant-per-generation rule to conservation and management. Conserv. Biol. 18, 332–343.
Weising, K., Nybom, H., Wolf, K., Kahl, G., 2005. DNA Finger Printing in Plants, second ed. CRC Press, Taylor and Francis.
White, G.M., Boshier, D.H., Powell, W., 1999. Genetic variation within a fragmented population of *Swietenia humilis* Zucc. Mol. Ecol. 8, 1899–1909.
Wolfe, A.D., Xiang, Q.Y., Kephart, S.R., 1998. Diploid hybrid speciation in Penstemon (Scrophulariaceae). Proc. Natl. Acad. Sci. U. S. A. 95, 5112–5115.
Wright, S., 1951. The genetical structure of population. Ann. Eugen. 15, 395–420.
Xia, T., Chen, S., Chen, S., Zhang, D., Zhang, D., Gao, Q., Ge, X., 2007. ISSR analysis of genetic diversity of the Qinghai-Tibet Plateau endemic *Rhodiola chrysanthemifolia* (Crassulaceae). Biochem. Syst. Ecol. 35, 209–214.
Xiao, L.Q., Ge, X.J., Gong, X., Hao, G., Zheng, S.X., 2004. ISSR variation in the endemic and endangered plant *Cycas guizhouensis* (Cycadaceae). Ann. Bot. 94, 133–138.
Yang, W.A., De-Oliveira, A.C., Godwin, I., Schertz, K., Bennetzen, J.L., 1996. Comparison of DNA marker technologies in characterizing plant genome diversity: variability in Chinese sorghums. Crop Sci. 36, 669–1676.
Yeh, F.C., Yang, R.C., Boyle, T., 1999. POPGENE Microsoft Window-based Freeware for Population Genetic Analysis: Release 1.32. University of Alberta, Edmonton, AB, Available <www.ualberta.ca/~fyeh/popgene_download.html/> (accessed on 01.04.15.).
Young, A., Boyle, T., Brown, T., 1996. The population genetic consequences of habitat fragmentation for plants. Trends Ecol. Evol. 11, 413–418.
Young, A.G., Clarke, G.M., 2000. Genetics, Demography and Viability of Fragmented Populations. Cambridge University, Cambridge.
Zietjiewicz, E., Rafalski, A., Labuda, D., 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20, 176–183.