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Genetic conservation of *Ficus bonijesulapensis* R.M. Castro in a dry forest on limestone outcrops

Jaqueline Fidelis Duarte [a, *], Dulcinéia de Carvalho [a], Fábio de Almeida Vieira [b]

[a] Departamento de Ciências Florestais, Campus Universitário, Universidade Federal de Lavras, Cx. Postal 3037, CEP 37200-000, Lavras, MG, Brazil

[b] Unidade Acadêmica Especializada em Ciências Agrárias, Universidade Federal do Rio Grande do Norte, RN 160, KM 03, Cx. Postal 07, CEP 59280-000, Macaíba, RN, Brazil

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ABSTRACT

Ficus bonijesulapensis is endemic to a seasonally dry forest on limestone outcrops and it is arranged in disjunct areas in the Cerrado and Caatinga Domains. Species of this genus are considered to be key plants in tropical forests, since they provide resources during periods of scarcity of other resources and, additionally, they contribute in a plant community's restoration. Therefore, the conservation of these species in their natural habitat contributes to the maintenance of the long-term population viability and their genetic diversity. We used nine ISSR primers to analyze the genetic diversity and the genetic spatial patterns of 15 populations of *F. bonijesulapensis*. We obtained 75 polymorphic bands, the expected heterozygosity (He) was 0.30 and AMOVA showed that most of the genetic diversity was found within populations (77%). The historical gene flow was 1.1 migrants and the Bayesian genetic structure assigned individuals genotypes to eight groups. However, there was not a spatial pattern of genetic variability according to a multivariate correlogram and as confirmed by Mantel's test ($r = 0.06$, $p = 0.68$). Eight management units (MU) were proposed and were aiming at the MU's ability to maintain minimally viable populations and a higher genetic variability.

1. Introduction

The deforestation of the Brazilian tropical rain forests is of an international concern and the increased number of studies available in the literature shows the profound interest in the conservation of these areas. However, most of the information comes from researches on the rain forests, while seasonal forests, even under such a large impact of destruction and fragmentation, are less studied (Pennington et al., 2000; Fuchs and Hamrick, 2010). Changes in temperature and in seasonal rainfall that occurred during the Quaternary age have influenced the history of vegetation and the climate throughout the South and Central Americas (Oliveira-Filho and Fontes, 2000), resulting in the disjunct distribution of seasonal forests (Prado, 2000). In Brazil, the Seasonally Dry Tropical Forests (SDTF) are found in the Caatinga and Cerrado Domains, and they are considered to be the remnants of vast semiarid vegetation that, during the Pleistocene epoch, might have spread and retracted over Central Brazil and parts of the Amazon (Prado and Gibbs, 1993; Pennington et al., 2004).

* Corresponding author. Tel.: +55 35 99365047.
E-mail address: jaquefdbio@hotmail.com (J.F. Duarte).

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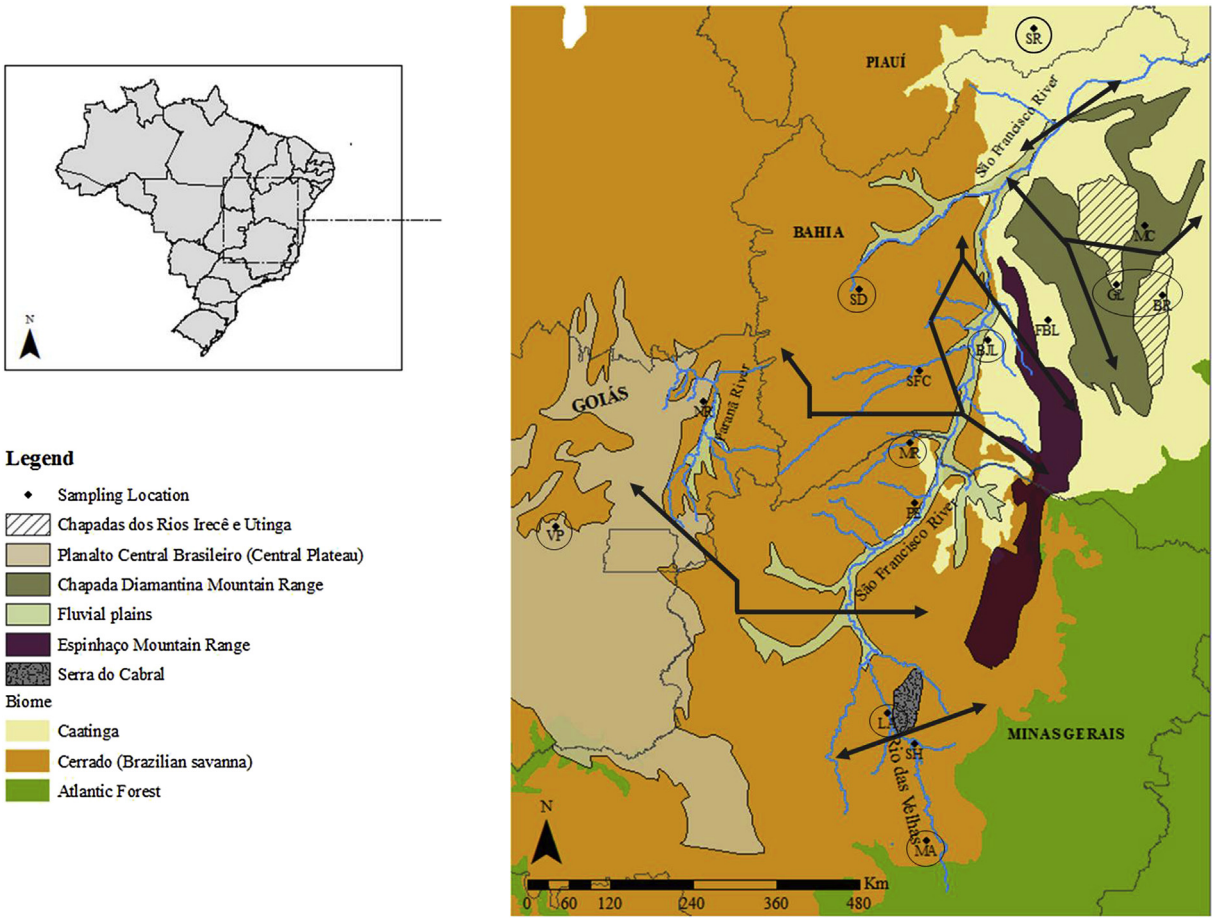

Fig. 1. Geographic location of the sampled populations of *Ficus bonijesulapensis* R. M. Castro (Moraceae). The genetic discontinuities among populations are represented by the black lines. The probably geographic barriers are represented too. The proposed MU's are identified with black circles.

The SDTF grow on different types of soil, such as rocky outcrops that, because they hold species that are able to survive extreme climates, and in particular edaphic conditions, are considered peculiar ecosystems and centers of diversity (Porembski and Barthlott, 2000; Felfili et al., 2007). *Ficus bonijesulapensis* R.M. Castro (Moraceae), a tree which information on its biology is scarce, is endemic to deciduous forests on outcrops (Castro and Rapini, 2006). However, the genus *Ficus* is widely known because of the mutual interaction between the fig tree and its pollinator wasps. Furthermore, another important role played by the fig tree is being a key resource, due to its asynchronous blooming during the whole year; they provide food for frugivorous animals, and in addition, its dispersion helps the process of the restoration of plant communities (Nason and Hamrick, 1997; Shanahan et al., 2001; Herre et al., 2008).

These characteristics highlight the importance of research regarding the vegetation that occurs on these outcrops, since they are areas that have been suffering an intense degradation due to exploitation mainly by miners (Silva and Scariot, 2004). In order to develop an effective conservation program, it is essential that genetic variability is maintained, because this is what enables the survival of a species in response to environmental changes and the long-term viability of its populations (Toro and Caballero, 2005). Thus, the use of molecular markers, based on the amplification of the DNA sequence, assists the estimative of population genetic parameters. Inter-Simple Sequence Repeat (ISSR) markers, in addition to being highly variable within a species, show a longer anchoring surface and support a higher annealing temperature, which leads to a greater reproducibility of amplified fragments and a robustness of the results (Bacchetta et al., 2011).

The genetic structure of a population is molded by the interaction of evolutionary factors, and is influenced by the population size, the species' life cycle and the gene flow. The latter is a result of both the pollinator and the disperser's efficiency to reach other populations and the isolation among them (Loveless and Hamrick, 1984; Nybom and Bartish, 2000). This isolation among populations may occur due to landscape barriers that can be roads, water bodies, mountain ranges, or even aspects such as humidity, temperature or chemical tolerance. The identification of these barriers against the gene flow is made with the aid of Landscape Genetics (Manel et al., 2003; Storfer et al., 2007). After identifying the spatial pattern of genetic structure, the so-called Management Units (MU's) can be suggested. These MU's would be populations that are

geographically distinct and genetically divergent, that, once identified, could improve the strategies of conservation *in situ* and aid the collection of samples for a germplasm bank (Diniz-Filho and Telles, 2002; Manel et al., 2003).

Since SDTF on rocky outcrops show a historic isolation, it is likely that *F. bonijesulapensis* shows a high genetic diversity among populations and the existence of natural barriers may be causing genetic disjunction among them. Furthermore, populations that are geographically closer are also expected to be more genetically similar. Therefore, in order to define strategies and delineate likely management units for the conservation of *F. bonijesulapensis*, this work aimed to answer the following questions: (1) What are the levels of genetic structure and diversity within and among the populations? (2) Is there any spatial autocorrelation pattern among the populations of *F. bonijesulapensis?*

2. Material and methods

2.1. Sampling

Leaf samples from the species *F. bonijesulapensis* were collected during an expedition throughout Central Brazil, and in the areas of Seasonally Dry Tropical Forests on limestone outcrops, which are distributed in patches in the following Brazilian states: Bahia, Goiás, Minas Gerais and Piauí (Fig. 1). Leaves from 15 locales were sampled, resulting in a total of 189 individuals. The locale of occurrence was defined as a population and the number of individuals found in each locale corresponded to the population size, i.e., samples were taken from all of the individuals in an area. Table 1 relates to the municipalities where the populations of *F. bonijesulapensis* were collected, with their respective identifications, geographical coordinates, and sample size.

2.2. DNA extraction and amplification with ISSR markers

The method to extract the DNA was previously described in Vieira et al. (2010). The reactions to amplify the DNA were carried out in a thermo cycler GeneAmp PCR System 9700. Thirty seven amplification cycles were performed, consisting of a 2-min initiation step at 94 °C, followed by a 15-s denaturation process at 94 °C, the annealing at 42 °C for 30 s, and the extension at 72 °C for 1 min. At the end, a 7-min extension step at 72 °C was carried out.

The total volume of each amplified sample was 12 μl: 2 μl of DNA was added to 10 μl of reaction mix [1.2 μl of buffer PCR 10× (500 mM of Tris–HCl pH 8.0; 200 mM of KCl; 2.5 mg/mL of BSA; 200 mM of Tartazine and 1% of Phycol), 1.2 μl of dNTP + $MgCl_2$ (dNTP at 2.5 mM; $MgCl_2$ at 25 mM), 0.15 μl of Taq polymerase (5 u/μl), 2 μl of primer (2 μM) and completed the final volume with ultra pure water]. The amplification products were submitted to electrophoresis on 1.5% agarose gel and photographed under UV light after Ethydium Bromide staining (5 mg/mL). 37 primers were used to identify which of them showed the best fragment profile.

2.3. Data analysis

The absence (0) or the presence (1) of the ISSR amplified fragments was registered to build the binary matrix used in the statistical analyses.

Table 1
Identification, municipality, geographical coordinates, sample size and estimates of diversity of the populations of *Ficus bonijesulapensis* R.M Castro (Moraceae).

Identification	Municipality	UF	Latitude/Longitude	n[a]	H_e[b]	I[c]	P (%)[d]
SR	São Raimundo Nonato	PI	08°59′33″S/42°40′54″W	14	0.20	0.38	68
BJL	Bom Jesus da Lapa	BA	13°02′35″S/43°16′35″W	18	0.21	0.38	71
GL	Gruta Lapa Doce	BA	12°19′53″S/41°36′23″W	13	0.21	0.37	68
BR	BR-242	BA	12°27′38″S/40°59′52″W	5	0.23	0.34	61
FBL	Mocambo	BA	12°47′22″S/42°29′37″W	3	0.09	0.12	21
MC	Morro do Chapéu	BA	11°33′06″S/41°14′14″W	13	0.23	0.46	83
SD	São Desidério	BA	12°22′52″S/44°56′58″W	2	0.05	0.07	12
SFC	São Félix do Coribe	BA	13°26′42″S/44°09′41″W	17	0.19	0.41	73
NR	Nova Roma	GO	13°50′45″S/46°57′96″W	14	0.25	0.48	85
VP	Vila Propício	GO	15°27′59″S/48°53′42″W	12	0.21	0.36	64
PE	Januária	MG	15°10′24″S/44°13′30″W	17	0.27	0.43	81
MR	Juvenília	MG	14°22′49″S/44°16′51″W	10	0.27	0.46	84
LA	Lassance	MG	17°54′00″S/44°34′51″W	11	0.32	0.49	91
MA	Matozinhos	MG	19°33′09″S/44°04′14″W	20	0.22	0.42	79
SH	Santo Hipólito	MG	18°17′41″S/44°13′11″W	20	0.26	0.52	89
Total				189	0.30	0.55	100

[a] n: sample size.
[b] H_e: expected heterozygosity.
[c] I: Shannon's index.
[d] P: percentage of polymorphic bands.

Table 2
ISSR primers selected to amplification of DNA of *Ficus bonjesuslapensis* R. M. Castro (Moraceae), their sequences of bases, number of amplified fragments and Polymorphic Information Content (PIC).

Primer	Sequence (5′–3′)	Bands	PIC
JOHN (AG)7-YC	AGA GAG AGA GAG AG**YC**	5	0.48
UBC 811 (GA)8-T	GAG AGA GAG AGA GAG A**C**	6	0.49
UBC 825 (AC)8-T	ACA CAC ACA CAC ACA C**T**	5	0.49
UBC 827 (AC)8G	ACA CAC ACA CAC ACA C**G**	7	0.49
UBC 835 (AG)8-YC	AGA GAG AGA GAG AGA G**YC**	8	0.48
UBC 841 (GA)8-YC	GAG AGA GAG AGA GAG A**YC**	11	0.49
UBC 842 (GA)8-YG	GAG AGA GAG AGA GAG A**YG**	7	0.33
UBC 844 (CT)8-RC	CTC TCT CTC TCT CTC T**RC**	8	0.46
UBC 857 (AC)8-YG	ACA CAC ACA CAC ACA C**YG**	18	0.47
Average		8	0.46

2.3.1. Genetic structure and diversity

The optimum number of polymorphic bands was analyzed by the program GENES (Cruz, 2001) using the bootstrap method. In order to be considered ideal to estimate the genetic diversity, the stress value indicates the adjustment between the original and the simulated matrixes and must be lower than 0.05. The parameters of genetic diversity: the percentage of polymorphic bands (PPB) and the expected heterozygosity (H_e), were calculated by the program TFPGA 1.3 (Miller, 1997), Shannon's information index (I), and the gene flow by POPGENE (v. 1.32) (Yeh et al., 1997).

The Polymorphic Information Content (PIC) was calculated using the formula PIC = 2Pi (1-Pi), where Pi is the frequency of the amplified polymorphic fragments and 1-Pi is the frequency of the null allele. The PIC indicated the efficiency of the ISSR primers to detect the polymorphism between two individuals, i.e., the capacity that each marker possesses to be found in two different states (absence/presence) in two plants taken randomly off the population. Thus, it can range between the values 0, for markers considered monomorphic, and 0.5, for those markers present in 50% of plants, and absent in the other 50% (Roldan-Ruiz et al., 2000).

The Analysis of Molecular Variance (AMOVA) was calculated by the program ARLEQUIN v. 3.1 (Excoffier et al., 2005), to determine the genetic variation within and among the populations.

2.3.2. Bayesian analysis of genetic differentiation

The genetic differentiation (θ^B) between the pairs of populations was calculated by means of Bayesian statistics in HICKORY v. 1.1 (Holsinger and Lewis, 2003). In this program, the value θ^B is calculated by the average of four different models: *full model*, $\theta^B = 0$, *free f* and $f = 0$. The values of the deviant information criterion (DIC) are compared in order to choose the model that best adjusts to the data, and the model with lowest DIC value is chosen (Holsinger and Wallace, 2004).

2.3.3. Genetic discontinuity and spatial patterns

The program BARRIER (Manni et al., 2004) was used in order to identify the discontinuity of genetic data in the geographical space. The 15 sampled populations were connected by Delaunay's triangulation method according to their geographical coordinates. The barriers were identified from Monmonier's algorithm (Manni et al., 2004). To evaluate the pattern of spatial structure between the populations, the coefficients were calculated from the correlation between the genetic distances (θ^B) and the matrices of spatial connectivity, using the program NTSYS v. 2.11x (Rohlf, 2000). To obtain each matrix, classes of geographical distances were defined so that it would be possible to maintain the same number of combinations among the populations in each class and, when this geographical distance between the pairs of populations is within these classes, the value of 1.0 is assigned. Mantel's test (10,000 permutations) was used in order to evaluate the significance of these matrix correlation coefficients, which were related to the increment of geographical distances, and thus resulting in a multivariate correlogram (Telles et al., 2007).

The management units (MU's) were suggested in accordance with the spatial pattern found and the minimum distance among these MU's would be based on the value of the interception on the horizontal axis (x-axis), according to the methodology proposed by Diniz-Filho and Telles (2002).

2.3.4. Bayesian genetic structure

A population genetic structure analysis was carried out based on the model of Bayesian grouping using the program STRUCTURE v. 2.3 (Pritchard et al., 2000). The individuals are probably grouped according to their genotypes in K populations, assuming that there are no linked loci. Besides the structuring, the model is able to identify the proportion of genotypes proceeding from the other groups. The set of parameters assumed the admixture model with correlated allele frequencies and 10 replications of 100.000 iterations were carried out after 50.000 burn-in repetitions, with K = 2–15. The number of populations was identified according to the ΔK method (Evanno et al., 2005) as implemented in the STRUCTURE HARVESTER program (Earl and vonHoldt, 2012). CLUMPP (Jakobsson and Rosenberg, 2007) was employed to align the results of 10 replicated runs for the highest ΔK value.

3. Results

3.1. Genetic structure and diversity

Thirty seven primers were tested, out of which nine were selected for resulting the best amplification profile to be codified. These primers produced 75 fragments, being that the minimum number of fragments per primer was five (JOHN and UBC 825) and the maximum was 18 (UBC 857). The average number of fragments was eight. The PIC values calculated for each primer ranged from between 0.33 and 0.49 and the average value was 0.46; this allowed the markers used in this study to be considered to be highly informative on the detection of polymorphism in the species (Table 2).

The number of polymorphic bands (63) was considered optimum to safely analyze the genetic diversity obtained by the ISSR primers. The observed stress value (0.049) was lower than 0.05 and the correlation was 0.97. The nine selected primers generated 75 polymorphic fragments (100%). The percentage of polymorphic bands observed in each population varied from 12% (São Felix do Coribe/BA) to 91% (Lassance/MG) (Table 1).

The expected heterozygosity (H_e), assuming the Hardy–Weinberg equilibrium, varied from 0.05 (SFC) to 0.32 (LA), with an average of 0.30, and Shannon's index (I) showed an average of 0.55, and varied from 0.07 (SFC) to 0.52 (MA) (Table 1). According to AMOVA, the majority of genetic diversity occurred within the populations (77%), a value considered average. Gene flow was 1.10, suggesting that the exchange of alleles among the populations is low.

3.2. Bayesian analysis of genetic differentiation

A Bayesian analysis was carried out by four different models and the value of θ^B was estimated for each model (Full: 3772, $f = 0$: 3793, $\theta^B = 0$: 5753, Free f: 3952). The *full model* showed itself to be the most adequate to estimate the genetic distance between the pairs of populations due to the lowest DIC value obtained.

Table 3 shows the genetic distances between the pairs of the populations obtained from θ^B. Populations SD and SFC showed the lowest value (0.02) and FBL and SFC showed the highest one (0.48). Seven classes of geographical distances were defined and the superior limits were 192 km, 304 km, 419 km, 515 km, 621 km, 749 km and 1200 km. According to Mantel's test, there was no significant correlation between the genetic distances and the matrixes of geographical distance ($r = 0.06$; $p = 0.68$), i.e. there was no spatial pattern on the genetic distances. This result was confirmed by the profile displayed on a multivariate correlogram where the horizontal axis was intercepted more than one time showing that there is no spatial pattern. Thus, each population could be identified as a management unit for the conservation and the access of genetic diversity for *F. bonijesulapensis.*

3.3. Spatial patterns and genetic discontinuity

The mapping of genetic distance θ^B by means of Delaunay's triangulation showed a series of genetic discontinuities (barriers) that separated even geographically close populations. Thus, the populations were separated as follows: (1) SR; (2) MC; (3) BR and GL; (4) FBL; (5) BJL; (6) SD and SFC; (7) MR, PE and NR; (8) VP and LA and (9) SH and MA, as can be observed in Fig. 1. The likely identified barriers to the gene flow were: (1) (2) (3) São Francisco river and different mountain ranges that belong to Chapada Diamantina and Chapadas dos Rios Irecê and Utinga that divide these populations; (4) Espinhaço mountain range and the fluvial plains near the São Francisco river; (5) Fluvial plains and São Francisco river; (6) River Paranã and the fluvial plains; (7) Mountain ranges from the Central Plateau; (8) River “das Velhas” and Serra do Cabral (Fig. 1).

Table 3
Matrix of genetic distance based on θ^B values (bottom) and geographic distance (Km) (top) between pairs of populations of *Ficus bonijesulapensis*. Minimum and maximum θB values are in bold.

Population	SR	MC	GL	BR	FBL	BJL	SFC	SD	MR	PE	NR	VP	LA	SH	MA
SR	**	324	388	426	420	453	518	449	621	704	712	985	1007	1043	1178
MC	0.11	**	95	104	194	276	381	415	455	515	672	935	789	812	936
GL	0.14	0.12	**	68	109	198	303	364	368	423	605	861	694	717	842
BR	0.18	0.22	0.11	**	167	256	360	430	414	460	665	916	714	732	851
FBL	0.39	0.39	0.38	0.26	**	89	195	271	262	323	498	752	608	637	767
BJL	0.33	0.35	0.28	0.20	0.12	**	106	196	184	257	409	663	555	590	725
SFC	0.29	0.26	0.25	0.14	**0.48**	0.29	**	145	104	191	307	557	495	537	676
SD	0.34	0.30	0.28	0.27	0.39	0.30	**0.02**	**	233	319	272	546	612	659	799
MR	0.31	0.31	0.27	0.21	0.17	0.08	0.18	0.19	**	88	296	511	391	433	573
PE	0.28	0.28	0.25	0.24	0.28	0.20	0.16	0.15	0.08	**	330	503	304	345	485
NR	0.25	0.26	0.24	0.17	0.18	0.16	0.18	0.19	0.08	0.04	**	274	516	573	703
VP	0.21	0.24	0.21	0.14	0.26	0.24	0.18	0.18	0.16	0.14	0.09	**	533	588	683
LA	0.28	0.30	0.26	0.12	0.17	0.20	0.20	0.24	0.13	0.19	0.10	0.05	**	58	191
SH	0.26	0.26	0.20	0.22	0.36	0.31	0.18	0.13	0.19	0.17	0.13	0.04	0.13	**	140
MA	0.18	0.22	0.17	0.12	0.30	0.25	0.04	0.12	0.17	0.14	0.14	0.06	0.12	0.05	**

3.4. Bayesian genetic structure

A Bayesian analysis of the genetic structure showed that the most likely number of clusters was K = 8 on the basis of the ΔK method.

4. Discussion

4.1. Genetic structure and diversity

The primers that were used to access the genetic diversity of *F. bonijesulapensis* were considered to be able to detect the polymorphism of the species, according to the value of PIC obtained. This value defines the efficiency that a class of markers has in order to detect the polymorphisms between two individuals (Rezende et al., 2009) and it can range from 0.0 to 0.5. Thus, values that are higher than zero (monomorphism) are able to detect polymorphism. Ikegami et al. (2009) analyzed varieties of *Ficus carica* by ISSR markers and classified them as being highly informative according to the PIC average value (0.39). Grativol et al. (2010) found the PIC average value of 0.26 per primer and considered that the set of ISSR primers that were used to analyze the species *Jatropha curcas,* were both efficient and suitable. When comparing these values with the PIC average that our research found, the ISSR markers used in this study were considered to be highly informative.

The number of polymorphic bands that are necessary to quantify the genetic variability of several studies carried out with ISSR markers is highly variable. For instance, Zhang et al. (2012) estimated 96 polymorphic bands for *Ormosia hosiei*, in *Sapindus trifoliatus* 185 bands were polymorphic (Mahar et al., 2013), and in *Butea monosperma* 71 bands were generated (Vashishtha et al., 2013). Therefore, a bootstrap analysis has been used in order to verify whether this number of polymorphic bands is sufficiently able to respond precisely to the estimative obtained in each study. Thus, the 75 polymorphic bands obtained for *F. bonijesulapensis* were considered to be sufficient to carry out the analyses.

Tree species show high levels of genetic diversity, which is a result of the interaction of factors such as high outcrossing rates and breeding systems that involve complex mechanisms of self-incompatibility and the pollination by animals (Bawa, 1974; Hamrick and Murawski, 1990; Cascante et al., 2002). The values of genetic diversity ($H_e = 0.30$, P = 100 and I = 0.55) obtained for the populations of *F. bonijesulapensis* were higher than in other studies with species of the same genus. Nason et al. (1998) observed that the levels of allozyme genetic diversity for the fig species studied had an average of 0.22 and that they were significantly higher than the population level estimates for other very low-density tropical trees. Nazareno and Carvalho (2009) observed that *Ficus arpazusa* showed high levels of genetic diversity ($H_e = 0.40$) and considered that this was due to the pollen dispersal distance and the mating system of *F. arpazusa*, which is an outcrossing species. For a study of the genetic structure of *Ficus pumila*, using microsatellite markers, both Chen et al. (2008) found $H_e = 0.43$ and Wang et al. (2009) obtained $H_e = 0.53$ for the same species located in different areas (island and continents) of China.

Commonly, it is expected that isolated populations have a low genetic diversity due to the small population size and a decreased gene flow. However, when a species has high dispersal ability, or if the population is colonized from different sources, this diversity may not be reduced (Frankham, 1997). Usually, outcrossing and perennial plant populations show higher values of diversity within populations than annual and autogamous plant populations (Nybom and Bartish, 2000). This result was also found for *F. bonijesulapensis* (77%), however, it is important to highlight that this was an average value, which may be a consequence of the low gene flow (1.1) among the populations, since genetic variability among and within the populations is a result of the interaction of different processes, such as geographical distribution, population fragmentation and isolation, reproductive systems, mutation, genetic drift, gene flow, and so forth (Nybom and Bartish, 2000).

In a studied carried out with the Mediterranean species *Juniperus phoenicea*, which has some of its populations dispersed over a wide geographical area, the highest genetic variability was found within the populations (76.5%) and the gene flow was estimated at 1.8. The authors postulated that the main barrier to the gene flow among the sampled populations was geographical isolation (Meloni et al., 2006). Nason et al. (1998) evaluated the effects of fragmentation, by using the paternity analysis technique, in populations of fig species that occur in low-density, in Panama. The results showed that the flowers are fertilized by pollen from a high number of different donors (18–55) and, due to the low-density in which these species occur, a dispersion distance of the wasps and the pollen was estimated to be 6 km–14 km in the populations. Shanahan et al. (2001) found a dispersion distance of 55 km and Zavodna et al. (2005) estimated a distance of 40 km for the fig wasps.

Fig trees are usually outcrossing and their pollination is known due to the mutual interaction with fig wasps (Agaonidae). Harrison (2003) suggested that these wasps are carried by the wind over long distances and, due to their olfactory ability, they are able to catch the odor exhaled by the fig inflorescences. It is important to note that the reproductive success of the fig trees depends directly on the presence of fig wasps, thus, the fragmentation may result in negative effects on the reproduction and on the genetics, both for the fig tree and for its pollinators (Nason and Hamrick, 1997).

The isolation of the outcrops, by itself, where *F. bonijesulapensis* occurs, is already a limiting factor for the species' dispersion. Furthermore, the sampled areas are surrounded by different landscapes such as the Cerrado and Caatinga vegetations, agricultural areas, mountain chains, different river basins, and urban agglomerates, what may hinder the movement of the pollinators and dispersers and thus contribute to the differentiation among populations.

4.2. Discontinuity, spatial patterns and Bayesian genetic structure

The establishment of programs for genetic conservation requires proposals that really ensure the maintenance of viable populations, i.e., that these populations show higher levels of genetic diversity, which may ensure the species' evolutionary potential. The evolutionary trajectory, the spatial organization, and the persistence of populations through time and space, are influenced by the gene flow pattern (Leclerc et al., 2008). Thus, it is important to detect the potential barriers that could limit the gene flow and to understand how these landscape elements influence genetic variability patterns. However, the direct observation of pollen and seed dispersal is usually logistically infeasible, so with a landscape genetics approach, it is possible to infer what habitat features hinder or even facilitate dispersion, and consequently, gene flow (Storfer et al., 2010).

Landscape genetics integrates population genetics, landscape ecology, and spatial statistics, to understand how genetic variability in natural populations is influenced by selection and environment variation (Manel et al., 2003; Segelbacher et al., 2010). Besides this approach, another proposal to improve the strategies of conservation *in situ* and *ex situ*, is to identify the spatial patterns of genetic structures. With the support of analyses of spatial autocorrelation, it is possible to identify those populations that are genetically divergent at a determined geographical distance and that can be considered management units for conservation. The values of correlation obtained for the *F. bonijesulapensis* populations were non-significant ($r = 0.06$; $p = 0.68$), i.e. the populations located at a far distance are not necessarily genetically divergent and can corroborate that the existence of geographical barriers led to the absence of correlation between the genetic and geographical distances (Haouari and Ferchichi, 2008). According to Diniz-Filho and Telles (2002), when there is no pattern of spatial correlation, the interception on the horizontal axis (X) can be adjusted to zero, since, even in a more precise spatial scale, it is not possible to detect autocorrelation. This interception on the horizontal axis would indicate from what geographical distance it would be possible to delineate genetically independent groups, and which would be considered management units. Therefore, in the present study, each sampled population could be defined as a management unit.

According to Koskela et al. (2013), the network of the units needs to reach all of the spatial genetic variation of the species studied, but they argue that it cannot be defined as a minimum number of units for all tree species. The minimum number of units should be determined species by species. Therefore, since it is necessary to define strategies of conservation that can be implemented, the definition of the management units for *F. bonijesulapensis* was carried out when considering both the highest values of intrapopulation genetic diversity and the number of individuals in the area. This is because each MU must be able to maintain minimum viable populations, and thus, avoiding the reduction of heterozygosity due to genetic drifts or inbreeding.

Another aspect that has been considered was the genetic boundaries detected; the rearrangement in eight clusters; and finally, the location of MU's on the extremes of the broad area of occurrence of the species that was covered. Thus, the areas that were chosen as management units can be seen in Fig. 1. The first unit would be located in São Raimundo Nonato (SR) in the Piauí State, followed by the area that comprises Gruta Lapa Doce (GL) and BR-242 (BR). The other units would be Bom Jesus da Lapa (BJL) and São Desidério (SD) in Bahia State, Juvenília (MR) in Minas Gerais State, Vila Propício (VP) in Goiás State, and Lassance (LA) and Matozinhos (MA) in the Minas Gerais State.

5. Implications for conservation

We believe that this work is the first to use ISSR primers to assess genetic variability in natural populations of *F. bonijesulapensis*. This species is endemic to the seasonal forests that occur on limestone outcrops that, nowadays, are being degraded, especially by limestone exploitation. Unfortunately, the destruction of these areas in itself causes a decrement in the population of fig trees, which blooming asynchronously, they are responsible for the survival of their pollinating wasps. Thus, it is essential to note that this species' specific interaction is so intrinsic that its disequilibrium can lead to the local extinction of both species.

Although the amount of genetic diversity as registered here was higher than others in the genus, the gene flow, which is responsible for maintaining the genetic diversity within populations, and hence, reducing the effects of inbreeding and genetic drift, was low. Then, it is fundamental to apply a conservation strategy that will ensure both the gene flow and the effective representation of genetic variability of the *F. bonijesulapensis* tree. Thus, the implementation of a management unit's network is a current proposal which contributes to the establishment of reserves or on the definition of sample areas for a germplasm bank. Therefore, conservation *in situ* of the *F. bonijesulapensis* tree ensures the permanence of many other species of the existent ecological interactions, and especially, those areas of limestone outcrops, that each day, are more threatened in Brazilian ecosystems. Another important factor to be considered is that the obtained results may help to comprehend how species that occur in naturally isolated areas behave, and also, how species located in those areas that were formerly continuous and suffered fragmentation, would be answering to this isolation.

Conservation genetic studies have been increasing over recent years in Brazil, but they still have a low priority in the investigation of the biodiversity of areas for conservation purposes. The management of target tree populations *in situ* should be a reality in this country, since we have a high level of forest diversity, where the interactions between the environment and species, as well as the competition within species, are the main factors responsible for the population's long-term evolutionary potential. We hope that this study attracts the attention of conservation biologists to use molecular tools as a basis for their researches in order that species genetic diversity can be ensured.

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References

Bacchetta, G., Fenu, G., Mattana, E., Zecca, G., Grassi, F., Casazza, G., Minuto, L., 2011. Genetic variability of the narrow endemic *Rhamnus persicifolia* Moris (Rhamnaceae) and its implications for conservation. Biochem. Syst. Ecol. 39, 477–484.
Bawa, K.S., 1974. Breeding systems of tree species of a lowland tropical community. Evolution 28, 85–92.
Cascante, A., Quesada, M., Lobo, J.J., Fuchs, E.A., 2002. Effects of dry tropical forest fragmentation on the reproductive success and genetic structure of the tree *Samanea saman*. Conserv. Biol. 16, 137–147.
Castro, R.M., Rapini, A., 2006. Four new species of Moraceae from Bahia, Brazil. Neodiversity 1, 13–20.
Chen, Y., Shi, M.M., Ai, B., Gu, J.M., Chen, X.Y., 2008. Genetic variation in island and mainland populations of *Ficus pumila* (Moraceae) in eastern Zhejiang of China. Symbiosis 45, 37–44.
Cruz, C.D., 2001. Programa GENES: versão Windows. UFV, Viçosa.
Diniz-Filho, J.A.F., Telles, M.P.d.C., 2002. Spatial autocorrelation analysis and the identification of operational units for conservation in continuous populations. Conserv. Biol. 16, 924–935.
Earl, D.A., vonHoldt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4 (2), 359–361. http://dx.doi.org/10.1007/s12686-011-9548-7.
Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol. Ecol. 14, 2611–2620. http://dx.doi.org/10.1111/j.1365-294X.2005.02553.x.
Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol. Bioinform. Online 1, 47–50.
Felfili, J.M., Nascimento, A.R.T., Fagg, C.W., Meirelles, E.M., 2007. Floristic composition and community structure of a seasonally deciduous forest on limestone outcrops in Central Brazil. Rev. Bras. Bot. 30, 611–621.
Frankham, R., 1997. Do island populations have less genetic variation than mainland populations? Heredity 78, 311–327.
Fuchs, E.J., Hamrick, J.L., 2010. Genetic diversity in the endangered tropical tree, *Guaiacum sanctum* (Zygophyllaceae). J. Hered. 101, 284–291.
Grativol, C., Lira-Medeiros, C.F., Hemerly, A., Ferreira, P., 2010. High efficiency and reliability of inter-simple sequence repeats (ISSR) markers for evaluation of genetic diversity in Brazilian cultivated *Jatropha curcas* L. accessions. Mol. Biol. Rep. 1–12.
Hamrick, J.L., Murawski, D.A., 1990. The breeding structure of tropical tree populations. Plant Spec. Biol. 5, 157–165.
Haouari, M., Ferchichi, A., 2008. Study of genetic polymorphism of *Artemisia herba-alba* from Tunisia using ISSR markers. Afr. J. Biotechnol. 7, 44–50.
Harrison, R.D., 2003. Fig wasp dispersal and the stability of a keystone plant resource in Borneo. Proc. R. Soc. B 270, S76–S79.
Herre, E.A., Jander, K.C., Machado, C.A., 2008. Evolutionary ecology of figs and their associates: recent progress and outstanding puzzles. Annu. Rev. Ecol. Evol. Syst. 39, 439–458.
Holsinger, K.E., Lewis, P.O., 2003. Hickory: a Package for Analysis of Population Genetic Data v1.1. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut, USA.
Holsinger, K.E., Wallace, L.E., 2004. Bayesian approaches for the analysis of population genetic structure: an example from *Platanthera leucophaea* (Orchidaceae). Mol. Ecol. 13, 887–894.
Ikegami, H., Nogata, H., Hirashima, K., Awamura, M., Nakahara, T., 2009. Analysis of genetic diversity among European and Asian fig varieties (*Ficus carica* L.) using ISSR, RAPD, and SSR markers. Genet. Resour. Crop Evol. 56, 201–209.
Jakobsson, M., Rosenberg, N.A., 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23, 1801–1806.
Koskela, J., Lefèvre, F., Schueler, S., Kraigher, H., Olrik, D.C., Hubert, J., Longauer, R., Bozzano, M., Yrjänä, L., Alizoti, P., Rotach, P., Vietto, L., Bordács, S., Myking, T., Eysteinsson, T., Souvannavong, O., Fady, B., De Cuyper, B., Heinze, B., von Wühlisch, G., Ducousso, A., Ditlevsen, B., 2013. Translating conservation genetics into management: Pan-European minimum requirements for dynamic conservation units of forest tree genetic diversity. Biol. Conserv. 157, 39–49.
Leclerc, E., Mailhot, Y., Mingelbier, M., Bernatchez, L., 2008. The landscape genetics of yellow perch (*Perca flavescens*) in a large fluvial ecosystem. Mol. Ecol. 17, 1702–1717.
Loveless, M.D., Hamrick, J.L., 1984. Ecological determinants of genetic-structure in plant-populations. Annu. Rev. Ecol. Syst. 15, 65–95.
Mahar, K., Rana, T., Ranade, S., Pande, V., Palni, L., 2013. Estimation of genetic variability and population structure in *Sapindus trifoliatus* L., using DNA fingerprinting methods. Trees 27, 85–96.
Manel, S., Schwartz, M.K., Luikart, G., Taberlet, P., 2003. Landscape genetics: combining landscape ecology and population genetics. Trends Ecol. Evol. 18, 189–197.
Manni, F., Guerard, E., Heyer, E., 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. Hum. Biol. 76, 173–190.
Meloni, M., Perini, D., Filigheddu, R., Binelli, G., 2006. Genetic variation in five Mediterranean populations of *Juniperus phoenicea* as revealed by inter-simple sequence repeat (ISSR) markers. Ann. Bot. 97, 299–304.
Miller, M.P., 1997. Tools for Population Genetic Analysis (TFPGA), Version 1.3. Department of Biological Sciences, Northern Arizona University, Arizona, USA.
Nason, J.D., Hamrick, J.L., 1997. Reproductive and genetic consequences of forest fragmentation: two case studies of neotropical canopy trees. J. Hered. 88, 264–276.
Nason, J.D., Herre, E.A., Hamrick, J.L., 1998. The breeding structure of a tropical keystone plant resource. Nature 391, 685–687.
Nazareno, A.G., Carvalho, D de, 2009. What the reasons for no inbreeding and high genetic diversity of the neotropical fig tree *Ficus arpazusa* ? Conserv. Genet. 10, 1789–1793.
Nybom, H., Bartish, I.V., 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspect. Plant Ecol. 3, 93–114.
Oliveira-Filho, A.T., Fontes, M.A.L., 2000. Patterns of floristic differentiation among Atlantic forests in Southeastern Brazil and the influence of climate. Biotropica 32, 793–810.
Pennington, R.T., Lavin, M., Prado, D.E., Pendry, C.A., Pell, S.K., Butterworth, C.A., 2004. Historical climate change and speciation: neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification. Philos. Trans. R. Soc. Lond. B Biol. Sci. 359, 515–538.
Pennington, R.T., Prado, D.E., Pendry, C.A., 2000. Neotropical seasonally dry forests and Quaternary vegetation changes. J. Biogeogr. 27, 261–273.
Porembski, S., Barthlott, W., 2000. Granitic and gneissic outcrops (inselbergs) as centers of diversity for desiccation-tolerant vascular plants. Plant Ecol. 151, 19–28.
Prado, D.E., 2000. Seasonally dry forests of tropical South America: from forgotten ecosystems to a new phytogeographic unit. Edinb. J. Bot. 57, 437–461.
Prado, D.E., Gibbs, P.E., 1993. Patterns of species distributions in the dry seasonal forests of South-America. Ann. Mo. Bot. Gard. 80, 902–927.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Rezende, R.K.S., Paiva, L.V., Paiva, R., Chalfun Junior, A., Torga, P.P., Masetto, T.E., 2009. Divergência genética entre cultivares de gérbera utilizando marcadores RAPD. Cienc. Rural. 39, 2435–2440.
Rohlf, F.J., 2000. NTSYSpc: Numerical Taxonomy and Multivariate Analysis System, 2.11 ed. Applied Biostatistics, New York.
Roldan-Ruiz, I., Dendauw, J., Van Bockstaele, E., Depciker, A., De Loose, M., 2000. AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). Mol. Breed. 6, 125–134.
Segelbacher, G., Cushman, S.A., Epperson, B.K., Fortin, M.-J., Francois, O., Hardy, O.J., Holderegger, R., Taberlet, P., Waits, L.P., Manel, S., 2010. Applications of landscape genetics in conservation biology: concepts and challenges. Conserv. Genet. 11, 375–385.
Shanahan, M., So, S., Compton, S.G., Corlett, R., 2001. Fig-eating by vertebrate frugivores: a global review. Biol. Rev. 76, 529–572.
Silva, L.A., Scariot, A., 2004. Comunidade arbórea de uma floresta estacional decídua sobre afloramento calcário na Bacia do rio Paraná. Rev. Arv. 28, 61–67.
Storfer, A., Murphy, M.A., Evans, J.S., Goldberg, C.S., Robinson, S., Spear, S.F., Dezzani, R., Delmelle, E., Vierling, L., Waits, L.P., 2007. Putting the 'landscape' in landscape genetics. Heredity 98, 128–142.
Storfer, A., Murphy, M.A., Spear, S.F., Holderegger, R., Waits, L.P., 2010. Landscape genetics: where are we now? Mol. Ecol. 19, 3496–3514.
Telles, M.P.C., Diniz, J.A.F., Bastos, R.P., Soares, T.N., Guimaraes, 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.
Toro, M.A., Caballero, A., 2005. Characterization and conservation of genetic diversity in subdivided populations. Philos. Trans. R. Soc. B-Biol. Sci. 360, 1367–1378.
Vashishtha, A., Jehan, T., Lakhanpaul, S., 2013. Genetic diversity and population structure of *Butea monosperma* (Lam.) Taub.- a potential medicinal legume tree. Physiol. Mol. Biol. Plants 1–9.
Vieira, F.A., Santana, J.A.D., Santos, R.M., Fajardo, C.G., Coelho, G.A.D., Carvalho, D., 2010. Métodos de extração de DNA e seleção de primers de cpDNA para *Ficus bonijesulapensis* (Moraceae). Rev. Caatinga 23, 69–74.
Wang, R., Ai, B., Gao, B.Q., Yu, S., Li, Y.Y., Chen, X.Y., 2009. Spatial genetic structure and restricted gene flow in a functionally dioecious fig, *Ficus pumila* L. var. *pumila* (Moraceae). Popul. Ecol. 51, 307–315.
Yeh, F.C., Yang, R.C., Boyle, T.B.J., Ye, Z.H., Mao, J.X., 1997. POPGENE, the User-friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Center, University of Alberta, Edmonton.
Zavodna, M., Arens, P., Van Dijk, P.J., Partomihardjo, T., Vosman, B., Van Damme, J.M.M., 2005. Pollinating fig wasps: genetic consequences of island recolonization. J. Evol. Biol. 18, 1234–1243.
Zhang, R., Zhou, Z., Du, K., 2012. Genetic diversity of natural populations of endangered *Ormosia hosiei*, endemic to China. Biochem.. Syst. Ecol. 40, 13–18.