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Spatial genetic structure of *Cavanillesia arborea* K. Schum. (Malvaceae) in seasonally dry Tropical forest: Implications for conservation

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ABSTRACT

Worldwide, Seasonally Dry Tropical Forests (SDTF) are among the least known and most degraded vegetation types. *Cavanillesia arborea* (Malvaceae) is an endemic dry forest tree that is bat-pollinated and the seeds are wind-dispersed. The present study sought to characterize the spatial genetic structure (SGS) in natural populations of *C. arborea* growing in SDTF fragments in Minas Gerais State, Brazil, using ISSR molecular markers. Seven ISSR primers generated 81 polymorphic loci in 175 individuals. In three populations (FU, MO and PC), we found random distributions of genotypes, while two populations (JAN and MV) showed SGS in their first distances classes (171 m and 17 m, respectively), with significant kinship (*Sp*) values (0.0189 and 0.0005 respectively; $P < 0.05$). *Sp* values for these populations indicate that the effects of kinship can be minimized by collecting seeds from distance classes beyond the species' first distance class. The continuous anthropogenic impacts on remnant tree populations, low species densities, and the observed genetic structure, all indicate the need to preserve large areas of dry forest vegetation for conservation purposes.

1. Introduction

Tropical ecosystems are rapidly being fragmented and altered as humans expand their activities into these environments. Deforestation has resulted in the formation of forest fragments that are the last refuge for many plant and animal species (Espírito-Santo et al., 2002). Genetic studies are becoming increasingly important in conservation programs as they can quantify genetic diversity distributions, the spatial structuring of genotypes, gene flow, and crossing rates, information that is essential for planners and resource managers (Moraes et al., 2005; Brandão et al., 2011).

Seasonally Dry Tropical Forests (SDTF), also known as Dry Forests, occur in areas with distinct annual dry and rainy seasons (Murphy and Lugo, 1986; Sanchez-Azofeifa et al., 2005; Vieira and Scariot, 2006). This phytophysiognomy occurs in

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disconnected patches throughout the Neotropical region, from Mexico and the Caribbean to southeastern/northeastern Brazil and the Chacos region of Argentina; currently, most dry forest remnants are found in South America (Pennington et al., 2006, 2000). Brazilian SDTFs occur in the central and northeastern regions of the country, including the northern part of Minas Gerais where they cover approximately 10% of the total area of the state (Prado and Gibbs, 1993). *Cavanillesia arborea* K. Schum. (Malvaceae) is an important species in these ecosystems.

Due to increasing anthropogenic pressure on these dry forests, and the restricted distribution of *C. arborea* (limited to only a few states in Brazil), studies that can aid the conservation and management of this species will also contribute to the preservation of its natural environment.

Five species of the genus *Cavanillesia* have been described, all them from the neotropical region (Fernández-Alonso, 2003). However, little is known about their ecology or genetics (Murawski et al., 1990; Díaz-Pérez et al., 2011). *C. arborea* looks like the Baobab (*Adansonia*) because of its large size, architectural beauty of their trunks and canopy, and generally long life span (Fig. 1). *C. arborea* can grow to over 35 m in height in fertile soils. Its flowers produce a disagreeable odor and large amounts of pollen. The flowers demonstrate nocturnal anthesis and are probably pollinated by bats, similar to many other species of Malvaceae (Gibbs and Semir, 2003); its seeds are winged and wind-dispersed (Vieira et al., 2008). Additional field observations are necessary to elucidate further aspects of the reproduction of *C. arborea* and the present study represents the first examination of the genetics of its populations.

Intrapopulation studies of spatial genetic structure (SGS) can detect groupings of related individuals by determining genetic frequencies or genotypes (Moraes et al., 2005). SGS within plant populations is primarily symptomatic of limited pollen and/or seed dispersal, the isolation of the plants in small groups, differential mortality, and the occupation of microhabitats (Epperson and Allard, 1989). Positive SGS (related individuals located close to each other within populations) may also indicate the occurrence of endogamous crossings (Sebbenn et al., 2011). This type of information is important for conservation management as it affects the minimum distance between individuals that should be considered while sampling (seed collections) for genetic improvement programs or for recuperating degraded areas in order to maintain the greatest possible genetic variability (Kelly et al., 2004; Cloutier et al., 2007; Brandão et al., 2011).

Several studies have successfully used the Inter-Simple Sequence Repeats (ISSR) technique to detect SGS (Chung et al., 2006; Brandão et al., 2011). Zietjiewicz et al. (1994) developed this technique, which is based on PCR amplification of DNA fragments present at amplifiable distances between two identical and repeated microsatellites that are oriented in different directions (Reddy et al., 2002).

In this study, we used ISSR molecular markers to characterize the micro-scale genetic structuring patterns found in five natural populations of *C. arborea* in northern Minas Gerais State, Brazil, in a region of Seasonally Dry Tropical Forests. Furthermore, we sought to determine the minimum distance necessary between trees for seed collection of *C. arborea* for genetic conservation programs.

2. Materials and methods

2.1. Location of the study site and sampling techniques

The study site is located in Seasonally Dry Tropical Forest in northern Minas Gerais State, Brazil, within the drainage basin of the São Francisco River and in the Espinhaço Mountain Range (Fig. 1). Individuals from five natural populations of *C. arborea* were sampled (Table 1).

Samples were taken from the trunks of *C. arborea* individuals in each study population, for a total of 175 sampled individuals (voucher number: 1969 – Herbarium of Montes Claros – HMC). The ability to extract DNA from the trunks of *C. arborea* was very useful as these trees are extremely tall and their leaves quite difficult to collect. All of the specimens sampled

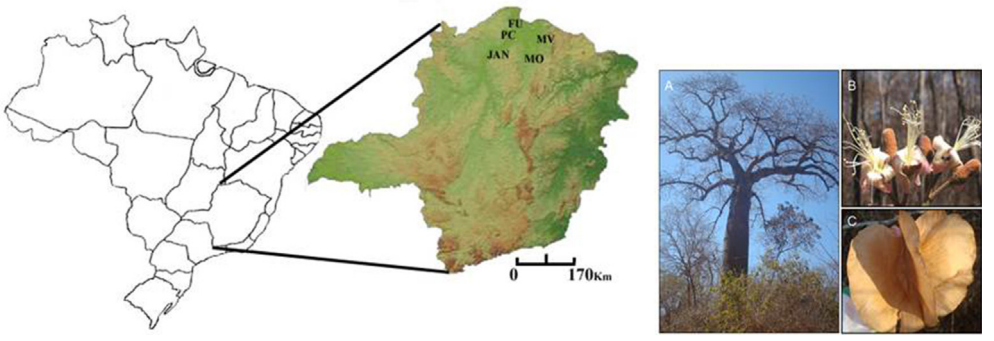

Fig. 1. Locations of the study populations of *Cavanillesia arborea* examined in northern Minas Gerais State, Brazil. JAN: Januária, FU: Mata Seca State Park (pop.1), PC: Mata Seca State Park (pop. 2), MO: Mocambinho, and MV: Mato Verde. (Image: IBGE). A) image of the *Cavanillesia arborea* tree in winter; B) flower; C): fruit. Images: Ferreira, M.F.M, 2008.

Table 1
List of the studied *Cavanillesia arborea* populations. Location, numbers of individuals sampled (*N*), altitude (*m*), and geographical coordinates of the study sites.

Population	Locality	*N*	Altitude (*m*)	Geographical coordinates
FU	Mata Seca State Park (pop.1)	37	488	14°50′39″ S 49°59′59″ W
PC	Mata Seca State Park (pop. 2)	44	478	14°49′23″S 47°53′26″ W
JAN	Januária, MG	41	461	15°13′12″ S 50°12′24″ W
MO	Mocambinho, MG	39	667	15°12′32″S 49°51′01″ W
MV	Mato Verde, MG	14	550	15°29′42″ S 42°53′47″ W

were geo-referenced using a Garmin Map 76CSX GPS. The collected materials were stored in 2 mL of 5% CTAB solution and subsequently transported to the Bio-Prospecting and Genetic Resources Laboratory at the University of Montes Claros, where they were stored at −20 °C until analysis.

Two populations separated by 7.87 km were sampled in the Mata Seca State Park. The first population (FU) included 37 adult individuals growing in a region of limestone outcrops; the second population (PC) had 44 individuals. The Mata Seca State Park, covering an area of 10,281 ha, was created in the year 2000 and is administered by the Minas Gerais State Forestry Institute.

The individuals in the Januária (JAN) population occupy a transition zone between Cerrado (Brazilian savanna) and SDTF, with many limestone outcrops occurring between agricultural and cattle grazing areas. Most of the native forest in this region has been replaced for agricultural purposes. The individuals sampled in this population were located in Permanent Preservation Zones on rural properties, although individual specimens of *C. arborea* were also found in pastures and patches of original vegetation that have been significantly modified by human activities. All of the adult individuals in this population were sampled ($N = 41$).

In the MO population, located in the municipality of Mocambinho, we also sampled all of the adult trees ($N = 39$). At this study site, the SDTF fragment is surrounded by irrigated orchards. During collection, we encountered grazing cattle as well as evidence of selective tree harvesting (principally *Myracrodruon urundeuva*, *Tabebuia ochracea* and *Caesalpinia pyramidalis*) to produce charcoal and/or fence posts.

The population at Mato Verde (MV) was located in a forest fragment surrounded by open pasture. All of the adult individuals at the site ($N = 14$) were sampled. This fragment is located in a semiarid region of Minas Gerais State that has been affected by accelerated processes of erosion caused by deforestation, elimination of riparian forests along watercourses, poor conservation of secondary roads, and predatory tourism in fragile environments.

2.2. DNA extraction and ISSR reactions

Genomic DNA was extracted from the trunks of *C. arborea* using the protocol proposed by Mogg and Bond (2003). PCR was performed in a final volume of 6.32 μL containing 10 mM Tris−HCl, 50 mM KCl, 2 mM $MgCl_2$, 100 μM of each deoxynucleotide (dATP, dTTP, dGTP, and dCTP), 0.4 μM of primer, 1 unit of Taq DNA polymerase, and 2 μL of DNA. The replication reactions were performed in a thermocycler using 40 cycles of 15 s at 94 °C, 30 s at 35 °C, and 1 min at 72 °C, followed by 7 min at 72 °C for complete extension of the amplification products. The amplified products were separated using horizontal electrophoresis in 1.2% agarose gel (p/v) at 120 V for 2.5 h. Visualizations and photo documentations of the amplification products were performed under ultraviolet light (UV) after staining with ethidium bromide (1 mg mL^{-1}).

2.3. Data analysis

Photographs of the gels were used to evaluate the presence or absence of amplified ISSR primer bands, generating a binary matrix that could be used to assess Spatial Genetic Structure (SGS). The SGS of the genotypes within each population were estimated using coancestry values and kinship coefficients between pairs of individuals within distance classes (Hardy, 2003) using the program SPAGEDI, version 1.2 (Hardy and Vekemans, 2002). Endogamy was considered to be zero in these analyses (Ritland, 1996). The kinship coefficient is described by the formula: $F_{ij} = (Q_{ij} - Q_m/1 - Q_m)$ (Rousset, 2002) where: F_{ij} represents the kinship coefficient of genetic coancestry between i and j; Q_{ij} is the probability that random samples of i and j would be identical due to parentage; and Q_m is the average probability that a random sampling made in the population would be identical due to parentage.

Confidence intervals were established using the standard errors of estimator measures obtained through jackknife resampling at a 95% probability level for the coefficient of estimated average coancestries for each distance class according to Hardy and Vekemans (2002). One thousand permutations were performed within each class to test for the occurrence of SGS and to determine its magnitude, as calculated by the formula (Vekemans and Hardy, 2004) $Sp = -b_{log}/(1-F_{ij(1)})$, where: b_{log} is

the slope of the regression curve of the coancestry coefficient; and $F_{ij(1)}$ is the average coancestry coefficient of the first distance classes (F_{ij}). We used the S_p values to compare the extent of SGS between the populations.

3. Results

The seven primers produced 81 polymorphic loci (Table 2) with the number of loci per primer ranging from 8 to 15 (average 11.57). As the sample size contained less than 50 individuals in each population, we considered loci polymorphic if the most common allele frequency was ≤95% (Nei, 1987).

In populations FU and MO, we noted an absence of significant genetic structuring ($P > 0.05$) with apparently random distributions of genotypes.

Significant SGS coancestry values were identified in the populations JAN, PC, and MV ($P < 0.05$) in some of their distance classes. However, the *Sp* value and the slope of the regression curve of the coancestry coefficient (b_{log}) indicated non-significant SGS values for the PC population. On the other hand, the JAN and MV populations showed S_P values that were significant and different from zero at a 95% probability level (Table 3).

The coancestry coefficients of the JAN population were significant for the first distance class (0–171 m) with a positive value of 0.037 ($P = 0.00$) as well as for the sixth distance class, with a negative value of −0.0445 ($P = 0.003$). These results indicate a spatial structuring where geographically closer individuals are genetically similar, indicating positive coancestry. Meanwhile, individuals in the sixth distance class (1558 m) that were more geographically distant had lower genetic similarities and, as such, negative coancestry was observed.

The correlogram of the PC population indicated low values of coancestry in the first distance class. On the other hand, positive and negative significant values of 0.033 ($P = -0.023$) and 0.015 ($P = 0.005$) were observed in the second and third classes, respectively. The *Sp* value showed an absence of SGS in this population; however, the slope of the regression curve of the coancestry coefficient (b_{log}) was not significant (Table 3).

The MV population demonstrated spatial genetic structure in the first distance class (between 0 and 17 m) with a coancestry value of 0.0289 ($P = 0.037$), indicating that geographically closer individuals were more genetically similar and showed positive coancestry.

4. Discussion

The S_p values of the five populations of *C. arborea* (Table 3) indicated significant spatial genetic structuring and b_{log} values only in the JAN and MV populations. These results are likely due to limited seed dispersal, which is aggravated by the degradation of the study areas and reduction in the numbers of surviving individuals, resulting in the formation of aggregates of *C. arborea* in the studied environments. The average S_p value observed for *C. arborea* ($S_p = 0.007$) is consistent with average values observed among outcrossing arboreal species ($S_p < 0.0126$; Vekemans and Hardy, 2004) and the low S_P values (between 0.0005 and 0.0189) are also very similar to those reported for other outcrossing arboreal species (Brandão et al., 2011; Vieira et al., 2010).

The contrasts seen between the populations in relation to SGS may be explained by the hypothesis that similarities between ecological conditions such as climate, soils, and altitude can lead to genetic similarities between individuals (Epperson and Allard, 1989; Barbará et al., 2008), and that pollen and seed dispersal between populations is often limited to matrix neighbors. As such, we expect that neighboring trees would demonstrate greater genetic similarity to each other than more distant individuals (Sokal and Menozzi, 1982; Hardy, 2003), as seen in the JAN and MV populations. There can also be physical limitations (such as habitat fragmentation) that could make random crosses within the population more probable between neighboring individuals. This would result in the formation of groups in a structured population (Sebbenn et al., 2011). Fruit dispersal by wind could likewise lead to spatial genetic structuring due to variations in the intensities of local winds, favoring barochoric dispersal near the mother tree.

Studies of SGS are important in projects considering the *in situ* conservation of natural populations and for sampling strategies, as this information can aid in identifying the true genetic diversity of a population or species (Epperson and Allard, 1989). Additionally, in the case of conservation management, this information can indicate the minimum sampling distance

Table 2
Names, sequences and numbers of fragments of seven ISSR primers used for amplifying *Cavanillesia arborea* DNA.

Primer name	Sequence (5′–3′)	N° of DNA fragments
UBC 850	GTGTGT GTG TGT GTGTYC	11
UBC 864	ATGATGATGATGATGATG	13
UBC 899	CATGGTGTTGGTCATTGTTCCA	15
TERRY (GTG)4-RC	GTG GTG GTG GTG RC	09
BECKY (CA)7-YC	CAC ACA CAC ACA CAY C	08
AW3 (GT)6-RG	GTG TGT GTG TGT RG	10
M1 CAA-(GA)5	CAA GAG AGA GAG A	15
Total		81

R = purine (A or G) and Y = pyrimidine (C or T).

Table 3
Spatial genetic structuring of five *Cavanillesia arborea* populations, indicating their kinship coefficients (F_{ij}) for the first distance class, b_{log}, S_p values, and the significance value (P) for each population.

Populations	F_{ij}	b_{log}	S_p	P
FU	0.0026	−0.0028	0.0028	0.508^{ns}
PC	−0.0056	−0.0005	0.0005	0.712^{ns}
JAN	0.0375	−0.0182	0.0189	0.000^{*}
MO	0.0083	−0.0029	0.0029	0.316^{ns}
MV	0.0289	−0.009	0.009	0.005^{*}

$^{*}P < 0.05$; ns = not significant.

for genetic improvement programs and seed collection, as it helps capture the greatest genetic variability (Cloutier et al., 2007; Jin et al., 2003; Kelly et al., 2004).

In the case of *C. arborea*, our study points to the presence of spatial genetic structure in the JAN population in the distance classes between 0 and 171 m (positive coancestry) and up to 1558 m (negative coancestry). The PC population showed negative coancestry in the 383 m distance class and positive coancestry at 500 m. Further, our results showed positive coancestry in the MV population in the distance class of 0–17 m.

Collecting seeds in populations demonstrating positive coancestry should be avoided to minimize the probability of close genetic relationships between specimens. Additionally, it is fundamental that conservation strategies focus on the preservation of large areas of dry forests as individuals of the same species often occur at considerable distances from each other (up to 3442 m). Information generated through studies of genetic diversity, genetic crossing systems, microscale spatial distributions of genotypes of regenerating individuals, and reproductive biology aid in our understanding of population dynamics and the genetic structure of the species occupying a given environment.

The data presented in this study can be used in efforts directed at preserving the highly fragmented Seasonally Dry Tropical Forests in northern Minas Gerais State, as very few studies have examined the genetics of their arboreal populations (Moreira et al., 2009). Therefore, in order to ensure high genetic variability in diaspore collection strategies in populations of *C. arborea*, a minimum distance of 171 m between trees should be respected in the JAN population, and 17 m in the MV population. Based on these results, the preservation of relatively large forest areas is necessary for the genetic conservation of this species. The creation of forest reserves, such as the Mata Seca State Park, is particularly important for the *in situ* maintenance of genetic diversity in populations of *C. arborea*.

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