Biochemical Systematics and Ecology 71 (2017) 147–154

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Fine-scale spatial genetic structure and gene flow in *Acrocomia aculeata* (Arecaceae): Analysis in an overlapping generation

Maircon Rasley Gonçalves Araújo [a], Afrânio Farias de Melo Júnior [a], Elytania Veiga Menezes [a], Murilo Malveira Brandão [a, *], Leide Gonçalves Cota [b], Dario Alves de Oliveira [a], Vanessa de Andrade Royo [a], Fábio Almeida Vieira [c]

[a] Universidade Estadual de Montes Claros, UNIMONTES, Postgraduate Program in Biotechnology, 39400-000, Montes Claros, MG, Brazil
[b] Universidade Estadual de Montes Claros, UNIMONTES, Postgraduate Program in Biological Sciences, 39400-000, Montes Claros, MG, Brazil
[c] Universidade Federal do Rio Grande do Norte, Department of Forest Engineering, Brazil

ARTICLE INFO

Article history:
Received 15 December 2016
Received in revised form 6 February 2017
Accepted 11 February 2017

Keywords:
Macaw palm
Mating system
Microsatellite markers
Outcrossing rate
Genetic conservation

ABSTRACT

This study aimed to assess the fine-scale spatial genetic structure (SGS) and gene flow among the developmental stages of *Acrocomia aculeata* (Arecaceae) in a natural population of the Brazilian savannah. The study was conducted in a 4.5 ha area using a population census composed of 72 adult and 144 juvenile plants. Six-hundred embryo samples were analyzed to investigate the gene flow of plant progenies. All individuals were genotyped using seven microsatellite *loci*. The microsatellite *loci* generated 10.1 alleles on average. Observed heterozygosity (H_o) was lower than expected heterozygosity (H_e) in all stages of development. The mean H_o and H_e values were 0.496 and 0.688, respectively. The *A. aculeata* population presented high genetic diversity despite its significant positive fixation index (0.279, $p = 0.001$). Significant SGS was observed for adults at the 2-m distance class and for juveniles at the 2 and 4-m distance classes. Average pollen flow distance was 105 m. High multilocus outcrossing rate was observed (1.2), indicating that the species is preferentially allogamous with evidence of self-incompatibility mechanisms. The *A. aculeata* population presents high potential for *in situ* and *ex situ* genetic conservation and germplasm collection.

1. Introduction

The intensive and uncontrolled exploitation of natural habitats has harmed the Brazilian tropical ecosystems, which are usually unprotected by the local legislation (Nazareno et al., 2012). Fragmentation of natural populations reduces the size of

* Corresponding author.
E-mail addresses: maicon.araujo@gmail.com (M.R.G. Araújo), afraniofariasdemelo@gmail.com (A.F. Melo Júnior), menezes.elytania@gmail.com (E.V. Menezes), murilomalveira@yahoo.com.br (M.M. Brandão), leide.cota@gmail.com (L.G. Cota), dario.aol@gmail.com (D.A. Oliveira), vanroyo31@yahoo.com.br (V.A. Royo), vieirafa@gmail.com (F.A. Vieira).
URL: http://www.ppgb.unimontes.br/, http://www.ppgb.unimontes.br/, http://www.ppgb.unimontes.br/, http://www.ppgb.unimontes.br/, http://www.ppgcb.unimontes.br/, http://www.ppgb.unimontes.br/, http://www.ppgb.unimontes.br/

http://dx.doi.org/10.1016/j.bse.2017.02.005
0305-1978/

the effective population, directly affecting the genetic diversity and reproduction and, consequently, the gene flow of plants (Aguilar et al., 2008). The immediate reduction of genetic diversity in populations is one of the effects of forest fragmentation. Thus fragmentation changes the genetic structure of plant population and increases inbreeding, which causes heavy losses to forest species populations (Young et al., 1996).

Due to ecological, historical, and evolutionary factors, the genotype distribution in a plant population may not occur randomly, creating spatial genetic structure – SGS (Vekemans and Hardy, 2004) and determining genetic diversity within and between populations, identified in the macro and micro scales (Slatkin, 1987). SGS can be defined as the genotype spatial distribution within a population, which results from different processes such as selection, demographic disorders, or environmental heterogeneity (Brandão et al., 2015; Vieira et al., 2012). The presence of SGS may affect genetic diversity most likely because of the creation of local family structures originated by restricted gene flow via seeds (Vekemans and Hardy, 2004).

Knowledge on SGS is important for application in conservation programs because it supports seed collection strategies for *in situ* and *ex situ* conservation projects, indicating the distance between genetically divergent trees in the population. In addition, it assists the management of natural populations, in which operating standards tend to affect genetic diversity (Doligez and Joly, 1997). In fact, genetic diversity is one of the necessary conditions for evolutionary change and, therefore, an essential component in the development of ecosystem conservation plans. The higher the inheritable genetic diversity of adaptive characteristics, the greater the phenotypic plasticity of individuals. Also, genetic diversity is important for maintaining the species in the long term, because knowing how this diversity is spread within and between populations allows for understanding the evolutionary history thereof and, thereby generating conservation and management strategies.

The study of gene flow refers to the movement of alleles within and between populations. Gene flow acts as an evolutionary force that homogenizes the genetic diversity within and between populations. However, with deforestation and the isolation of populations owing to fragmentation, low gene flow rates reduce genetic diversity and, eventually, decrease the adaptation of plant species (Dick et al., 2008). Currently, determination of pollen and seed dispersion within tree populations has been effectively performed based on highly polymorphic genetic markers, such as microsatellites (Bittencourt and Sebbenn, 2007). Nowadays, this tool is widely used in genetic maps, paternity tests, gene flow, population analysis, and ecological and conservation biology studies (Bittencourt and Sebbenn, 2007).

The species *Acrocomia aculeata* (Jacq.) Lodd. ex Martius (Arecaceae) is native to the tropical forests and is present from the south of Mexico to the south of Brazil, Paraguai and Argentina. Macaw Palm is mainly found in semi-deciduous forest valleys and slopes. Regarding the botanic aspects, its stipe may reach 10–15 m in height and 20–30 cm in diameter (Motta et al., 2002). It is a monoicous species, with spadix inflorescence, and unisexual flowers (Henderson et al., 1995). They are visited by bees of the genus Trigona, which collect pollen from male flowers and pollinate the female flowers (Henderson et al., 1995). Coleoptera are also among the main pollinators, including the families *Curculionidae*, *Nitidulidae* and *Escarabaeidae* (Henderson et al., 1995). Nevertheless, no detailed studies on this species mating system are available in the specific literature. *A. aculeata* dispersion is facilitated by large fruit production, which are consumed by several animal species such as macaws, capybaras, tapirs, rheas, etc.

The *A. aculeata* is among the palm trees of greater geographic dispersion in Brazil, with natural populations occurring in almost all Brazilian territory. Because its adapts well to arid and semi-arid regions, macaw palm can be cultivated in marginal areas or in areas with certain degree of environmental degradation, contributing to the reduction of environmental impacts arising from the successive deforestation to plant oilseeds.

Unplanned extractivism results in the fragmentation and loss of local populations, increasing inbreeding and decreasing genetic diversity. In view of this, conservation programs aiming to maintain the existing levels of genetic diversity have been implemented (Nazareno et al., 2012). This fact highlights the importance of research on population genetics for the conservation of species, which is one of the objectives of this work. Production of data to support this aspect determines the success of any conservation program, because they depend on knowledge on the genetic diversity within the species.

The following hypotheses were considered in the present study: 1) genetic diversity between *Acrocomia aculeata* progenies is lower than that between adult individuals because the area of study is degraded and fragmented, and these factors contribute to the reduction of genetic diversity over the generations; 2) the *A. aculeata* population presents high gene flow and outcrossing rate considering that allogamy is suggested as a means of reproduction for this species according to the expectations for tree species (Murawski, 1995; Collevatti et al., 2001); 3) fine-scale spatial genetic structuring is present in different distance classes considering that outcrossings between related individuals have been reported by studies on the mating system of several natural populations of tropical tree species (Carneiro et al., 2007), reinforcing the hypothesis of fine-scale SGS, and that part of the gene flow is restricted to the vicinity of mother trees. In view of this, this study aimed to assess the fine-scale spatial genetic structure and gene flow among different developmental stages of an *A. aculeata* population in the transition area of Brazilian savanna and semi-arid region, in the north of Minas Gerais state.

2. Material and methods

2.1. Study site, sampling, and DNA extraction

The study was conducted in a 4.5 ha area located in the rural area of the municipality of Montes Claros, State of Minas Gerais, Brazil (16°48′04″ S; 43°55′99″ W) (Fig. 1). The north of Minas Gerais state is characterized by a transition between domains of Brazilian savannah and Stational Deciduous Tropical Forest (SDTF); it is a priority area for conservation (Cavalcanti

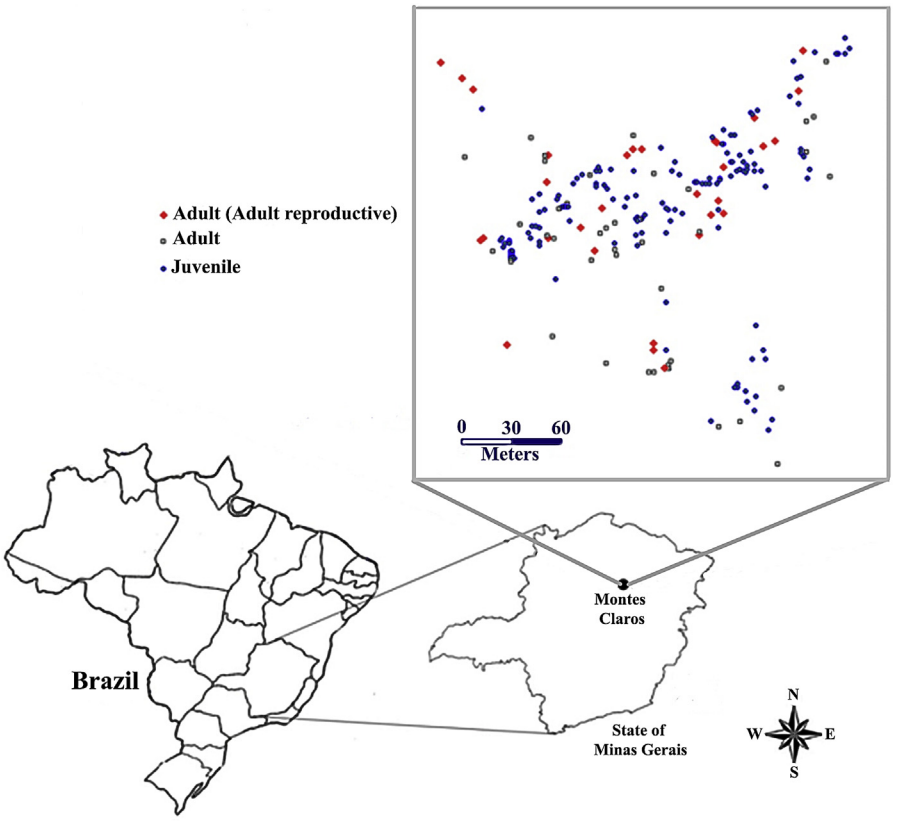

Fig. 1. Geographical distribution of *Acrocomia aculeata* individuals in the sampled population in the municipality of Montes Claros, State of Minas Gerais, Brazil.

and Joly, 2002). The physiognomic classification of the area is dirty field, based on field observations and according to the description by Bittencourt and Sebbenn (2007). The method proposed by Santos and Vieira (2005) was used to assess the state of preservation of the sampled area. Assessment was conducted with regard to the presence of livestock, fire, and selective logging. Scores vary from 1 to 5, with 1 referring to sites with the worst state of conservation and 5 to sites with the best state of conservation. The area was classified as 2, because it shows few and scattered vegetation individuals, presence of livestock, recent fire history, in addition to plant species selective logging due to pasture and planting.

A census of the population was conducted in a demarcated rectangular plot (4.5 ha). Outside the collection area, the shortest distance between individuals of the species was 1.5 km. Small fragments were found in the adjacent areas. There was an unpaved road west of the collected area. Results of the census showed 72 adult plants in fruiting phase, with height ranging from 3 to 22 m and 144 juvenile plants below the height, measuring between 0.1 and 1.5 m. All individuals were identified, sampled, and georeferenced. In order to study the gene flow among generations 20 mature fruits were collected from each of 30 adult individuals, selected by simple random sampling, for progeny analysis, totaling 600 samples for embryo DNA extraction. Thus the sampling size was 816 individuals. During the field collection, leaf samples were placed in plastic bags with silica gel, and subsequently stored at −80 °C. The DNA of the adult and juvenile individuals was extracted according to the CTAB 5%. DNA from the embryos was extracted according to the protocol proposed by Mogg and Bond (2003).

2.2. Analysis of microsatellites

Seven microsatellite *loci* (Aacu07, Aacu10, Aacu12, Aacu26, Aacu30, Aacu32, Aacu35), developed to *Acrocomia aculeata* by Nucci et al. (2008), were used to genotype all the sampled individuals. The amplification reactions were performed in 15 μL volume, with 1X buffer (10 mM Tris-HCl, pH 8.0, 50 mM KCl), 0.7 μM of each primer, 250 μM of each dNTPs, 1 unit of Taq DNA polymerase (Phoneutria, BR), 0.25 mg of BSA, 1.0 mM of $MgCl_2$, and 9 ng of DNA. Amplifications were performed using a Veriti 96 Well Thermal Cycler (Applied Biosystems, CA) under the following conditions: initial denaturation at 94 °C for 5 min (one cycle), 94 °C for 1 min, 45 °C to 56 °C for 1 min (according to each pair of primers), 72 °C for 1 min (35 cycles), and 72 °C for 30 min (one cycle). The amplified products were submitted to electrophoresis in an automatic DNA sequencer, model ABI 3500 (Applied Biosystems, CA) and dimensioned by comparison with a known length DNA: Liz 600 (Applied Biosystem, CA) using the software Gene Mapper v. 4.1 (Applied Biosystems, CA). Analyze of gametic disequilibrium was performed using the software Fstat 2.9.3.2 (Goudet, 2002), with Bonferroni correction (Rice, 1989). Detection of errors in genotyping was verified

by Micro Checker 2.2.3 software (Van Oosterhout et al., 2004). The frequency of the null alleles was calculated by the method of Dempster et al. (1977), using FreeNA software (Chapuis and Estoup, 2007).

2.3. Genetic diversity and structure

Genetic diversity was estimated by the number of alleles by *loci* (*A*), observed heterozygosity (H_o), expected heterozygosity (H_e) under the Hardy-Weinberg equilibrium, and fixation index (f) using the software Genetic Data Analysis - GDA 1.1 (Lewis and Zaykin, 2001).

To verify whether the developmental stages (adult, juvenile, and progenies) presented differences, the genetic structure was estimated based on the Weir and Cockerham (1984) coancestry coefficient. The coefficients were estimated from the variance of allelic frequencies of individuals, with F (total fixation index), f (fixation index), and θ (differentiation between progenies, juveniles, and adults), using the software Fstat 2.9.3.2, with 1000 randomizations and Bonferroni correction (Goudet, 2002).

The genetic differentiation between the developmental stages analyzed was also estimated by the G_{ST} (Goudet et al., 1996) index, a genetic differentiation measurement adequate for mutation rates of microsatellite *loci*; test significance was estimated by genotype randomization among the samples to obtain the G *log-likelihood* statistics (Hedrick, 2005). After analysis using the Fstat software, the alleles that presented allelic frequency lower than 0.05 were considered as rare.

2.4. Fine-scale spatial genetic structure

The fine-scale spatial genetic structure (SGS) analysis of the adult and juvenile genotypes was performed based on the estimated coancestry coefficient (kinship) between pairs of individuals for 10 classes of distances, not defined *a priori* (Hardy, 2003) using the software SPAGeDI, version 1.2 (Hardy and Vekemans, 2002). Based on the mean standard error of the estimates, obtained by permutation of individuals, confidence intervals of the mean estimated coancestry coefficient for the distance classes were constructed at 95% probability according to Hardy and Vekemans (2002). The occurrence of SGS was tested by 1000 permutations within each class, and its magnitude was calculated using the formula: $Sp = -b_{log}/(1 - F_{ij(1)})$ (Hardy and Vekemans, 2002); where b_{log} is the regression slope of the coancestry coefficient and $F_{ij(1)}$ is the coancestry coefficient (F_{ij}) of the first class of distance for all *loci*. The *Sp* values were used to compare the extension of the SGS between adult and juvenile individuals.

2.5. Gene flow and mating system

Paternity of individuals, with known mother, was inferred using the software Cervus 3.0.3 (Marshall et al., 1998). The software inferred the paternity through the likelihood ratio expressed as LOD *scores* (likelihood ratio log) considering two possible pollen donors. The following parameters were used: 10,000 cycles, 1.0% error rate, 99% strict level of confidence for distribution generation, and critical values of the difference in LOD *scores* between two most closely related individuals.

The gene flow through pollen was assessed by analysis of progeny structure using the software TWO-GENER (Austerlitz and Smouse, 2001). The principle of this method is to estimate the differentiation of allelic frequency among the crossed-pollen set (Φ_{ft}) between different mother trees in the study area. The Φ_{ft} parameter is calculated by analysis of molecular variance (AMOVA) (Excoffier et al., 1992) as a male gamete (pollen) intra-class correlation within mother trees, where σ^2_a is the variance in pollen frequency between trees and σ^2_d is the variance in pollen frequency within trees. The Φ_{ft} parameter was estimated on gamete haplotypes of pollen captured for an average of 30 mother trees assessed.

The outcrossing rates for all *loci* (tm) and for a single *locus* (ts) were estimated and compared between the open pollination families. The single locus outcrossing rate concerns the crossover between unrelated individuals (ts). The difference between the multi*locus* and single *locus* outcrossing rates (tm - ts) indicates the occurrence of outcrossing between related individuals. The analysis was based on the mixed-mating model using bootstrapping (Ritland, 2004), whose model is based on progeny analysis in which the detection of the non-maternal allele in the progeny genotype indicates the occurrence of outcrossing. Standard error was obtained for each parameter based on 1000 permutations.

3. Results

3.1. Genetic diversity and structure

After a Bonferroni correction no locus analyzed showed significant deviation of the Hardy-Weinberg equilibrium. The estimation of genotyping errors revealed a presence of null alleles in the Aacu12 locus, only for the juvenile samples (frequency 10%). On the other hand, there was no presence of dropout and/or stutters for the analyzed alleles. The frequency of errors was adjusted using the Oosterhout estimator which considers as non-amplified samples such as degraded DNA or human error, for example.

The seven microsatellite *loci* assessed for the *A. aculeata* population indicated an overall average of 10.1 alleles for the plant developmental stages (Table 1). Observed heterozygosity (H_o) was lower than expected heterozygosity (H_e) in all

Table 1
Genetic diversity of the *Acrocomia aculeata* population individuals based on seven microsatellite *loci*; $\hat{A}$ - mean number of alleles per developmental stage; H_e - expected heterozygosity; H_o - observed heterozygosity; *f* - fixation index, SE (95%): standard error.

Developmental stage	$\hat{A}$	*He*	*Ho*	*f*
Adult	9.4	0.733	0.516	0.296*
Juvenile	10.4	0.680	0.575	0.155*
Progeny	10.5	0.651	0.398	0.388*
Average	10.1	0.688	0.496	0.279*
-SE (95%)	3.33	0.144	0.101	0.058
+SE (95%)	3.49	0.131	0.098	0.096

*$p = 0.001$, significant values; SE = Standard Error.

developmental stages, resulting in high, significant fixation indices *(f)* for adults, juveniles, and progenies. The *f* overall mean value was significantly positive for the developmental stages of *A. aculeata* (Table 1).

The fixation index (*f*) and the total fixation index *(F)* were significant for the *loci* Aacu07, Aacu10, Aacu12, Aacu26, and Aacu30, with Aacu07 and Aacu12 showing the highest values (Table 2). Aacu32 and Aacu35 presented non-significant values, and Aacu35 showed negative *f* and *F* values. However, the mean estimates of *f* (0.338) and *F* (0.385) for the developmental stages were significant ($p = 0.001$) (Table 2).

Population differentiation was verified by the values of the θ and G_{ST} indices, which were significant for most *loci* ($p = 0.001$), except for *locus* Aacu12. The means for the θ and G_{ST} indices were 0.072 and 0.036, respectively (Table 2). Genetic differentiation was significant between the different stages of development analyzed, with lower differentiation between adults and juveniles ($\theta = 0.026$) and higher differentiation between juveniles and progenies ($\theta = 0.089$).

3.2. Fine-scale spatial genetic structure (SGS)

Significant SGS was found for juveniles and adults, with significant positive coancestry coefficient values ($p < 0.05$). The genotypes are non-randomly distributed among adults in the 2-m class (*Fij* = 0.02; $p = 0.005$) and among juveniles in the 2 (*Fij* = 0.04) and 4-m (*Fij* = 0.013) classes. Significant negative kinship was observed among juvenile individuals ($p < 0.05$) in the 7, 9, 15, and 17-m distance classes. The *Sp* values evidenced genetic structuring for adult (Sp = 0.014, P < 0.001) and juvenile individuals (Sp = 0.014, P < 0.001), with kinship coefficient in the first distance class (*Fij)* of 0.021 and 0.046, respectively.

3.3. Gene flow and mating system

Gene flow through pollen estimated by the mean distance between the pollen donor and the reproductive palm tree was 105.2 m, with minimum and maximum distances of 0.5 m and 287 m, respectively. Eighty-three percent of the possible pollen donors in the study area participated in the pollination of trees. However, 30% of the adult reproductive palm trees were not pollinated by plants from the same area. Paternity was inferred for 10.5% of the assessed progenies, and it is likely that palm trees were pollinated by plants from other areas.

Pollen flow mean distance (gene flow) was 105 m, with pollen dispersion standard deviation of 0.7 m. The estimated mean spatial distance between mothers was 10.51 m and the density of reproductive trees by hectare (*d*) was 16. The global Φ_{ft}, which measures the crossed-pollen genetic divergence across reproductive palm trees, was 0.353. Considering $\Phi_{ft} > 0$, it is possible to conclude that the area presents restricted pollen dispersion for this species.

The multi*locus* outcrossing rate (t_m) was high (1.2), with standard deviation of ±0.13, indicating that the species is preferably allogamous. However, the single *locus* outcrossing rate (t_s) was lower (0.701), with a standard deviation of ±0.275, and significantly different from t_m considering a confidence interval of 95% probability. The difference between the multi*locus* and single *locus* outcrossing rates (t_m - t_s) was 0.499, with standard deviation of ±0,278. Considering the 95% confidence interval obtained for the difference t_m - t_s, this estimate is statistically significant.

4. Discussion

4.1. Genetic diversity and structure

The *Acrocomia aculeata* population assessed presents high genetic diversity despite its significant positive fixation index (*f*). It was possible to observe that the genetic diversity tends to reduce over the generations probably due to inbreeding. In addition, genetic diversity among adults is relatively higher than among juveniles, which, in turn, is higher than among progenies. The effects of fragmentation can lead to loss of genetic diversity, generally, in the generations established after these processes, due to reduction of the effective population size and other factors (Goverde et al., 2002). The results obtained for *A. aculeata* are similar to those reported by other studies on microsatellite markers, which show a tendency of lower genetic diversity for progenies compared with that for adult trees (Resende et al., 2011; Sebbenn et al., 2011).

Table 2
Genetic structure of *Acrocomia aculeata* based on variance analysis of allelic frequencies and allele size for seven microsatellite *loci*. *f* (fixation index), *F* (total fixation index), θ (stage differentiation) and G_{ST} (genetic differentiation between stages).

Locus	Repeated reason	*f*	*F*	θ	G_{ST}
Aacu07	(GA)13	0.657	0.696	0.114	0.053
Aacu10	(AG)16	0.475	0.544	0.13	0.063
Aacu12	(TC)20	0.574	0.582	0.018*	0.007*
Aacu26	(AC)13 (AG)14	0.344	0.383	0.059	0.033
Aacu30	(CA)18	0.338	0.351	0.02	0.016
Aacu32	(TG)22	0.04*	0.057*	0.018	0.009
Aacu35	(TG)20	−0.215*	−0.057*	0.115	0.058
For all *loci*	–	0.338	0.385	0.072	0.036

*$p > 0.001$, non-significant values.

In recent years the *Acrocomia aculeata* has shown a promising palm for extractivism. However, the irrational exploitation of natural resources, environmental degradation and climate change undermine the survival of their populations in the long term. Environmental degradation and fragmentation of natural populations can influence the behavior of agents that promote the gene flow of tree species (Resende et al., 2011). Similarly, the pollinators of *A. aculeata,* which are beetles of the families *Curculionidae*, *Nitidulidae* and *Escarabaeidae* (Henderson et al., 1995), have most likely been affected by the low preservation index of the study area. Moreover, dispersion of *A. aculeata* fruits, facilitated by several animal species such as macaws, capybaras, tapirs, rheas, etc., may also be affected by environmental changes, because the presence of these animals is little observed in open and fragmented areas (Motta et al., 2002).

Low genetic differentiation was observed for the stages analyzed ($\theta = 0.072$; $G_{ST} = 0.036$), because according to Frankham et al. (2002), only differentiation values higher than 0.15 are considered indicative of significant differentiation between populations. The pairwise differentiation indicated that progenies are further away from the other stages. As noted, the presence of alleles observed in progenies and not in adults may be due to pollen from outside the sampled area, as the group of progenies presents different alleles from those found in other stages.

The presence of exclusive alleles in these natural populations is important to decision making on conservation of genetic resources Kalinowski (2004). Therefore, having observed unique alleles in different stages of development of the *A. aculeata* population, *in situ* and *ex situ* genetic conservation strategies are essential to preserve and ensure the maintenance of the genetic diversity of this species.

4.2. Fine-scale spatial genetic structure (SGS)

Several studies on tree species have shown that seed dispersion is the main cause of SGS (Lacerda et al., 2008; Bittencourt and Sebbenn, 2007; Carneiro et al., 2007). This process reveals that the seeds are removed from the mother plant vicinity to “safe” distances; where predation and competition are lower (Howe and Miriti, 2004). Thus the dispersing agents - gravity, water, wind, or animals - may directly influence the maximum distance of seed dispersion (Guariguata and Pinard, 1998). However, the results of this study show the presence of genetic structures in short distance classes (2 and 4 m) among adult and juvenile individuals, indicating that seed dispersion occurs in distances adjacent to the parent.

In spite of the literature reports of zoochorous dispersion (Motta et al., 2002), the SGS in *A. aculeata* adults and juveniles suggests a predominantly barochoric seed dispersion, with fruits falling next to the reproductive palm trees and remaining there until germination, without the action of other vectors. The mean spatial distance of seed dispersion (105 m) in the population studied was lower than that observed in other forest species, whose seeds are dispersed primarily by zoochory (*Copaifera langsdorffii* - Sebbenn et al., 2011), autochory (*Araucaria angustifolia* – Carneiro et al., 2007), and barochory (*Quercus salicina* – Howe and Miriti, 2004). The genetic structure in the *A. aculeata* population, associated with other biological features of the species such as reproduction method and dispersion standards, must be considered when preparing strategies for genetic material collection of the species for conservation.

4.3. Gene flow

Not all plants in the reproductive stage were pollinated by trees from the same area; individuals from other areas have probably contributed to the pollination of these plants. In fact, through field observation, reproductive individuals of *A. aculeata* were found at a distance of approximately 1.5 km. Generally, anthropized areas present low diversity and density of species, causing the immigration of pollination vectors to new foraging areas (Llorens et al., 2012). Thus the pollen dispersal radius in a population may vary depending on pollination.

The presence of unique alleles in juveniles and progenies confirms that the *A. aculeata* population sampled is not isolated reproductively, reinforcing that the gene flow originates from individuals from other areas. Adult individuals also presented unique alleles, which may indicate that not all alleles present in this developmental stage were transmitted to the progenies.

Regarding density analysis, a high rate (16 breeding plants/ha^{-1}) was observed for the species studied, taking into account that this density is considered high for populations of tropical species with more than 10 plants/ha^{-1} (Degen et al., 2004). A

positive relation between the highest population density and the allelic dispersion in short distances is observed when the density variation is > 5 plants/ha^{-1} (Silva et al., 2008). In addition, the average pollen dispersion distance found (105 m) confirms the restricted gene flow, which has also been reported for other species (Carneiro et al., 2007; Llorens et al., 2012). In fact, the contemporary gene movement at short distances for *A. aculeata* is consistent with the presence of fine-scale spatial genetic structure in adults and juveniles.

The preservation of gene flow among populations is of relevant importance for the maintenance of genetic diversity indexes. Thus, the conservation of forest fragments and the fauna involved in the processes of pollination and dispersal of *A. aculeata* becomes critical action.

4.4. Reproductive system

The *Acrocomia aculeata* has a high multilocus outcrossing rate (1.200), suggesting an inbreeding preference with evidence of self-incompatibility, as described by Scariot et al. (1991). The multi*locus* outcrossing rates for *A. aculeata* are similar to those found in other preferably allogamous tropical tree species such as *Quararibea asterolepis* (t_m = 1.008) (Murawski, 1995), *Caryocar brasiliense* (t_m = 1.000) (Collevatti et al., 2001), *Hymenaea courbaril* (t_m = 1.002) (Lacerda et al., 2008), and *Euterpe edulis* (t_m = 0.900–0.980) (Conte et al., 2008). The high difference value between *Acrocomia aculeata* multi*locus* and single *locus* outcrossing rates indicates inbreeding, which may justify the high fixation index found.

The paternity correlation, which indicates the percentage of plants generated through bi-parental outcrossing, was high, suggesting a non-random crossed-pollination process. The species presents restricted pollen dispersion in the studied area as the values of crossed-pollen genetic divergence between reproductive plants were greater than zero. The causes of bi-parental outcrossing may be attributed to the behavior of pollinators, systematically visiting nearby trees, and to the asynchrony in flowering trees (Sun and Ritland, 1998). In fact, a lack of flowering was observed in the field, where reproductive plants showed different phenological stages, with predominance of buds or flowers on separate plants. The variation in the degree of synchrony between individuals within the same population changes the effective distance between flowering trees, and may limit the actual number of mattings between individuals. Environmental variations can also affect the behavior and/or density of pollinating animal populations, leading to changes in the outcrossing rates of species in different populations and times (Murawski, 1995).

Funding

This work was supported by the Petrobras: Programa de Formação de Recursos Humanos (PRH-PB 225) grant number 6000.0071556.11.4, Fundação de Amparo à Pesquisa do Estado de Minas Gerais for Endogovernamental Demand - grant number CRA - DEG-19-13 – Research and Technological Development Incentive scholarships (BIPDT) to D.A. Oliveira – Process number CBB-BIP-00049-14, and Researcher Visiting scholarships (BPV) to M.M. Brandão – grant number BPV-00654-15.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2017.02.005.

References

Aguilar, R., Quesada, M., Ashworth, L., Herrerias-Diego, Y., Lobo, J.A., 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Mol. Ecol. 17, 5177–5188. http://dx.doi.org/10.1111/j.1365-294X.2008.03971.x.

Austerlitz, F., Smouse, P.E., 2001. Two-generation analysis of pollen flow across a landscape. II. Relation between Φ_{fi}, pollen dispersal and interfemale distance. Genetics 157, 851–857.

Bittencourt, J.M., Sebbenn, A.M., 2007. Patterns of pollen and seed dispersal in a small, fragmented population of the wind-pollinated tree *Araucaria angustifolia* in southern Brazil. Heredity 99, 580–591. http://dx.doi.org/10.1038/sj.hdy.6801019.

Brandão, M.M., Vieira, F.A., Nazareno, A.G., Carvalho, D., 2015. Genetic Diversity of Neotropical Tree *Myrcia Splendens* (Myrtaceae) in a Fragment-corridor System in the Atlantic Rainforest. Flora, Jena. http://dx.doi.org/10.1016/j.flora.2015.07.006.

Carneiro, F.S., Sebbenn, A.M., Kanashiro, M., Degen, B., 2007. Low interannual variation of mating system and gene flow of *Symphonia globuliferain* the brazilian amazon. Biotropica 39 (5), 628–636. http://dx.doi.org/10.1111/j.1744-7429.2007.00314.x.

Cavalcanti, R.B., Joly, C.A., 2002. Biodiversity and conservation priorities in the cerrado region, cap. 18. In: Oliveira, P.S., Marquis, R.J. (Eds.), The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna. Columbia University Press, New York.

Chapuis, M.-P., Estoup, A., 2007. Microsatellite null alleles and estimation of population differentiation. Chicago Mol. Biol. Evol. 24 (3), 621–631.

Collevatti, R., Grattapaglia, D., Hay, J.D., 2001. High resolution microsatellite based analysis of the mating system allows the detection of significant biparental inbreeding in *Caryocar brasiliense*, an endangered tropical tree species. Heredity 86, 60–67.

Conte, R., Mantovani, A., Reis, M.S., Vencovsky, R., 2008. Genetic structure and mating system of *Euterpe edulis* Mart. Populations: a comparative analysis using microsatellite and allozyme markers. J. Hered. 99, 476–482. http://dx.doi.org/10.1093/jhered/esn055.

Degen, B., Bandou, E., Caron, H., 2004. Limited pollen dispersal and biparental inbreeding in *Symphonia globulifera* in French Guiana. Heredity 93, 585–591. http://dx.doi.org/10.1038/sj.hdy.6800560.

Dempster, A.P., Laird, N.M., Rubin, D.B., 1977. Maximum likelihood from incomplete data via the EM algorithm. London J. R. Stat. Soc. 39 (1), 1–38. Apr. 1977.

Dick, C.W., Hardy, O.J., Jones, F.A., Petit, R.S., 2008. Spatial Scales of Pollen and seed - mediated Gene flow in tropical rain forest trees. New York Trop. Plant Biol. 1, 20–33. http://dx.doi.org/10.1007/s12042-007-9006-6.

Doligez, A., Joly, H.I., 1997. Mating system of *Carapa procera* (Meliaceae) in the French Guiana tropical forest. Am. J. Bot. 84, 461–470.

Excoffier, L., Smouse, P.E., Quattro, J.W., 1992. Analysis of molecular variance inferred from metric distance among DNA haplotypes: applications to human mitochondrial DNA restriction data. Genetics 131, 479–491.
Frankham, R., Ballou, J.D., Briscoe, D.A., 2002. Conservation Genetics. Cambridge University Press, Cambridge UK, p. 617.
Goudet, J., Raymond, M., De-Meeus, T., Rousset, F., 1996. Testing Differentiation in Diploid Populations. Genetics 144, 1933–1940.
Goudet, J., 2002. Fstat, a Program to Estimate and Test Gene Diversities and Fixation Indices (Version 2.9.3.2). http://www.unil.ch/izea/softwares/fstat.html (accessed 16.07.15).
Goverde, M., Schweizer, K., Baur, A.E., 2002. Small-scale habitat fragmentation effects on pollinator behaviour: experimental evidence from the bumblebee *Bombus veteranus* on calcareous grasslands. Biol. Conserv. 104, 293–299.
Guariguata, M.R., Pinard, M.A., 1998. Ecological knowledge of regeneration from seed in neotropical Forest trees: implication for natural Forest management. For. Ecol. Manag. 112, 87–99.
Hardy, O.J., Vekemans, X., 2002. SPAGeDi 1.2: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol. Ecol. Notes 2, 618–620. http://www.ulb.ac.be/sciences/lagev/software.html (accessed 16.07.09).
Hardy, O.J., 2003. Estimation of pairwise relatedness between individuals and characterization of isolation by distance processes using dominant genetic markers. Mol. Ecol. 12, 1577–1588.
Hedrick, P.W., 2005. A standardized genetic differentiation measure. Evolution 59, 1633–1638. http://dx.doi.org/10.1111/j.0014-3820.2005.tb01814.x.
Henderson, A., Galeano, G., Bernal, R., 1995. Field Guide to the Palms of the Americas New Jersey. Princepton University, pp. 166–167.
Howe, H.F., Miriti, M.N., 2004. When seed dispersal matters. BioScience 54, 651–660.
Kalinowski, S.T., 2004. Counting alleles with rarefaction: private alleles and hierarchical sampling designs. Conserv. Genet. (5), 539–543.
Lacerda, A.E.B., Kanashiro, M., Sebbenn, A.M., 2008. Long-pollen movement and deviation of random mating in a low-density continuous population of a tropical tree *Hymenaea courbaril* in the Brazilian Amazon. Biotropica 40, 462–470. http://dx.doi.org/10.1111/j.1744-7429.2008.00402.x.
Lewis, P.O., Zaykin, D., 2001. Genetic Data Analysis: Computer Program for the Analysis of Allelic Data. Version 1.0. http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php (accessed 16.07.09).
Llorens, T.M., Byrne, M., Yates, C.J., Nistelberger, H.M., Coates, D.J., 2012. Evaluating the influence of different aspects of habitat fragmentation on mating patterns and pollen dispersal in the bird-pollinated banksia sphaerocarpa var. caesia. Mol. Ecol. 21 (2), 314–328. http://dx.doi.org/10.1111/j.1365-294X.2011.05396.x.
Marshall, T.C., Slate, J., Kruuk, L.E.B., Pemberton, J.M., 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol. Ecol. 7, 639–655. http://dx.doi.org/10.1046/j.1365-294x.1998.00374.x.
Mogg, R.J., Bond, J.M., 2003. A cheap, reliable and rapid method of extracting high quality DNA from plants. Mol. Ecol. Notes 3, 666–668.
Motta, P.E.F., Curi, N., Oliveira-Filho, A.T., Gomes, J.B.V., 2002. Ocorrência da macaúba em Minas Gerais: relação de atributos climáticos, pedológicos e vegetacionais. Pesqui. agropecuária Bras. 37 (7), 1023–1031.
Murawski, D.A., 1995. Reproductive biology and genetics of tropical trees from a canopy perspective. In: Lowman, M.D., Nadkarmi, N.M. (Eds.), Forest Canopies. Academic Press, New York, pp. 457–493.
Nazareno, A.G., Feres, J.M., Carvalho, D., Sebbenn, A.M., Lovejoy, T.E., Laurance, W.F., 2012. Serious new threat to brazilian forests. Conserv. Biol. 26, 5–6. http://dx.doi.org/10.1111/j.1523-1739.2011.01798.x.
Nucci, S.M., Azevedo-Filho, J.A., Colombo, C.A., Priolli, R.H.G., Coelho, R.M., Mata, T.L., Zucchi, M.I., 2008. Development and characterization of microsatellites markers from the macaw. Mol. Ecol. Resour. 8 (1), 224–226. http://dx.doi.org/10.1111/j.1471-8286.2007.01932.x.
Resende, L., Ribeiro, R., Lovato, M., 2011. Diversity and genetic connectivity among populations of a threatened tree (Dalbergia nigra) in a recently fragmented landscape of the Brazilian Atlantic Forest. Dordrecht Genetica 139 (0), 1159–1168. http://dx.doi.org/10.1007/s10709-011-9618-5.
Rice, W.R., 1989. Analyzing tables of statistical tests. Washington Evolution 43 (1), 223–225.
Ritland, K., 2004. Multilocus Mating System Program MLTR: Version 2.4. Disponível emhttp://genetics.forestry.ubc.ca/ritland/programs. University of British Columbia, British Columbia.
Santos, R.M., Vieira, F.A., 2005. Structural Analysis of the Arboreal Component of Three Areas of Savannah in Different Stadiums of Conservation, in Três Marias Count. Minas Gerais state, brazil. Cerne, pp. 399–408.
Scariot, A.O., Lleras, E., Hay, J.D., 1991. Reproductive biology of the palm *Acrocomia aculeata* in Central Brazil. Biotropica 23, 12–22.
Sebbenn, A.M., Carvalho, A.C., Freitas, M.L.M., Moraes, S.M.B., Gaino, A.P.S.C., Silva, J.M., Jolivet, C., Moraes, M.L.T., 2011. Low levels of realized seed and pollen gene flow and strong spatial genetic structure in a small, isolated and fragmented population of the tropical tree *Copaifera langsdorffii* Desf. London Heredity 106 (1), 134–145. http://dx.doi.org/10.1038/hdy.2010.33.
Silva, M.B., Kanashiro, M., Ciampi, A.Y., Thompson, I., Sebbenn, A.M., 2008. Genetic effects of selective logging and pollen gene flow in a low-density population of the dioecious tropical tree *Bagassa guianensis* in the Brazilian Amazon. For. Ecol. Manag. 255, 1548–1558. http://dx.doi.org/10.1016/j.foreco.2007.11.012.
Slatkin, M., 1987. Gene flow and the geographic structure of natural populations. Science 236, 787–792.
Sun, M., Ritland, K., 1998. Mating system of yellow starthistle (*Centaurea solstitialis*), a successful colonizer in North America. Heredity 80, 225–232.
Van Oosterhout, C., et al., 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes, Oxf. 4 (3), 535–538.
Vekemans, X., Hardy, O.J., 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. Mol. Ecol. 13 (4), 921–935.
Vieira, F.,A., Fajardo, C.G., Carvalho, D., Reis, C.A.F., Souza, A.M., 2012. Fine-scale genetic dynamics of a dominant neotropical tree in the threatened Brazilian Atlantic Rainforest. Tree Genet. Genomes 8, 1191–1201.
Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution 38, 1358–1370.
Young, A., Boyle, T., Brown, T., 1996. The population genetic consequences of habitat fragmentation for plants. Trends Ecol. Evol. Oxf. 11 (10), 413–418.