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Mating System and Genetic Composition of the Macaw Palm (*Acrocomia aculeata*): Implications for Breeding and Genetic Conservation Programs

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Abstract

Acrocomia aculeata (Arecaceae), a palm endemic to South and Central America, is a potential oil crop. Knowledge of the mating system of this species is limited to its reproductive biology and to studies using molecular markers. The present study analyzed genetic diversity between its developmental stages and determined its prevailing mating system in order to support genetic conservation and breeding programs. We tested 9 microsatellite markers in 27 mother trees (adult plants) and 157 offspring (juvenile plants) from the southeastern region of Brazil. Heterozygosity levels differed between the 2 studied life stages, as indicated by the fixation index of adult and juvenile trees, suggesting that selection against homozygotes occurs during the plant life cycle. The mating system parameters analyzed indicate that *A. aculeata* is predominantly outcrossing (allogamous). However, its low levels of selfing suggest that there is individual variation with regard to self-incompatibility, which can be a survival strategy in isolated or fragmented habitats. Deviations in variance effective size were detected because of high mating rates among relatives and correlated matings. These findings indicate that the main source of inbreeding results from biparental inbreeding in the population and that the progenies are predominantly composed of full-sibs. The information provided by this study on the ecology and reproduction dynamics of *A. aculeata* should be useful to both breeding and genetic conservation programs, allowing the development of more precise mathematical models and the estimation of the appropriate number of mother trees for seed collection.

Subject areas: Conservation genetics and biodiversity; Reproductive strategies and kinship analysis
Key words: *Acrocomia aculeata*, Arecaceae, inbreeding, macauba, macaw palm, outcrossing

Introduction

Palms are a key element of the structure and functioning of tropical terrestrial ecosystems (Zona and Henderson 1989; Galetti et al. 1999; Salm et al. 2005; Genini 2009; Barfod et al. 2011). The Arecaceae family has approximately 2450 species (Barfod et al. 2011), including *Acrocomia aculeata* (Jacq.) Lodd. ex Martius, an endemic plant of South and Central America (Henderson et al. 1995) commonly known as the macaw palm or macauba. Being one of the most widespread palm in the Neotropics, this palm is of ecological importance as a pioneer in secondary succession and as food source for the vertebrate fauna. This species has also inspired much interest of the industry, as an alternative renewable energy source (Pires et al. 2013).

The high productivity potential of *A. aculeata* (over 6200 kg oil ha^{-1}, Wandeck and Justo 1988), added to its dispersion throughout tropical America, put this species in a strategic position as the primary alternative to oil palm (*Elaeis guineensis*), which is currently the world's only oil-producing palm. Additionally, the oils extracted from the pulp and kernel of *A. aculeata* meet the prerequirements of both the biofuel and pharmaceutical industries (Bora and Rocha 2004; Hiane et al. 2005; Coimbra and Jorge 2012; Ciconini et al. 2013; Berton et al. 2014). Lastly, its fruit processing generates a variety of solid residues that can yield co-products useful in other energy sectors, including charcoal, second-generation ethanol, and bio-kerosene (Silva et al. 1986; Gonçalves et al. 2013; Lanes et al. 2014). Thus, *A. aculeata* has the potential to become a mainstay of sustainable tropical agriculture. However, to fulfill its potential, the species must be domesticated.

Because of its phenotypic plasticity, *A. aculeata* often thrives in areas with adverse edaphoclimatic conditions, including those with low water supply, high irradiance, and low fertile soils (Motoike and Kuki 2009; Abreu et al. 2011). This innate resilience foretells that *A. aculeata* can be cultivated in degraded pasture or abandoned croplands, which reduce the need for acquiring new agricultural lands. Additionally, future plantations of the palm can be set up under various agricultural systems, including intercrops or silvopastoral practices (Ciconini et al. 2013). These sensible cultivation models of *A. aculeata* may promote forest protection, CO_2 sequestration, and biodiversity in degraded areas (Lanes et al. 2014).

Acrocomia aculeata ($2n = 30$, Abreu et al. 2011) is a monoecious species, with numerous male and female flowers gathered in a spadix inflorescence and marked protogyny (Scariot et al. 1991). Although protogyny is a reproductive mechanism that favors cross-pollination, the presence of several inflorescences in a single individual enables selfing. This fertilization mode may represent an adaptive strategy that allows this species to persist in new habitats, especially in the absence of pollinators or in isolated small populations. Therefore, under limited circumstances, *A. aculeata* may produce offspring that comprise various degrees of relatedness. The downside is that successive generations of inbreeding increases homozygosity leading to inbreeding depression, that is, decline in the mean value of phenotype that is generally fitness related (Falconer 1989). As a result, the ability of the population to thrive under environmental pressures, such as the loss and fragmentation of its habitat, is compromised (Scariot et al. 1991; Dudash and Fenster 2000; Markert et al. 2010; Nazareno and Reis 2012).

The type of mating system and its variations within a species are determined by reproductive and ecological factors (Henderson 1986; Murawski and Hamrick 1991; Núñez-Avellaneda et al. 2005; Fuchs and Hamrick 2011; Khanduri et al. 2013) or by inherent genetic factors such as self-incompatibility (Goodwillie et al. 2005). Several hermaphroditic tree species are self-incompatible or partially self-incompatible (Bawa 1974; Zapata and Arroyo 1978; Bullock 1985), and variations in the self-incompatibility system are common among Arecaceae species, as has been shown with *Euterpe edulis* (Gaiotto et al. 2003), *Astrocaryum aculeatum* (Ramos et al. 2011), *Oenocarpus bataua* (Ottewell et al. 2012), and *Butia eriospatha* (Nazareno and Reis 2012).

The mating system plays an important role in the genetic composition of a population, ultimately dictating the degree of genetic recombination in the next generation. To date, research on the mating system of *A. aculeata* has been limited to studies of its reproductive biology (Scariot et al. 1991) and the application of Microsatellites, or simple sequence repeats (SSR) molecular markers in natural populations (Nucci et al. 2008; Abreu et al. 2012). Studies of inbreeding and genetic structure parameters as well as estimates of heterozygosity in the life stages of *A. aculeata* would be useful in elucidating its reproductive strategies, ecology, and diversity.

In this study, we determined whether heterozygosity levels differ between the juvenile and adult stages and whether mixed mating or outcrossing predominates in this species. Additionally, we estimated mating system parameters that might be used to support decision making in conservation and breeding programs. To our knowledge, this is the most comprehensive work on the mating system of *A. aculeata* and the first to report differences in heterozygosity between life stages.

Materials and Methods

Germplasm Bank Description and Sample Collection

The Macauba Active Germplasm Bank (BAG–Macauba) is located on the Araponga Experimental Farm in the municipality of Araponga, State of Minas Gerais, in the southeastern region of Brazil (latitude 20°40′1″S; longitude 42°31′15″W; altitude of ~1000 m). The climate of the region is characterized by rainy summers and dry winters and has a Köppen classification of Cwb. BAG–Macauba is an official repository registered by the Brazilian Board of Genetic Heritage (# 084/2013-SECEX/CEGEN) and is one of the largest collections of *A. aculeata* germplasm in South America. The maintenance and improvement of the germplasm collection are performed by the Macaw Palm Breeding Program at the Plant Science Department of the Universidade Federal de Viçosa (UFV—Brazil).

Currently, the repository holds a total of 253 maternal families, representing almost all Brazilian regions. New plants are continuously added to the collection and are all from seed propagation. Past and future maternal adult plants (referred to as mother trees) are georeferenced using a GPS with ±7 m accuracy (Garmin, Atchison, KS) in their native habitats. During *A. aculeata* fruiting season, mature fruits are collected for seeds, which are extracted and pregerminated using a protocol developed specifically for the species (Motoike et al. 2007). The plantlets are grown in the nursery house, and when they reach at least 10 months old they are added to the germplasm bank. In the BAG–Macauba the juvenile plants (referred to as offspring or progeny) are managed according to established field practices (Pires et al. 2013). The offspring that come from the same mother tree are referred to as a "maternal family."

Plant Material, Genomic DNA Extraction, and Microsatellites Analysis

The study examined 19 georeferenced *A. aculeata* mother trees (adult plants) and 157 of their offspring (juvenile plants) growing

at BAG–Macauba (an average of 8.3 offspring per mother tree). In addition, 8 nonrelated adult plants, also georeferenced, were included in the analysis. All 27 adult plants were from natural populations in southeast Brazil (Figure 1). DNA samples of adult plants were obtained from stipe tissue, whereas those of juvenile plants were from leaflets. In both cases, DNA extraction was performed following the modified hexadecyltrimethylammonium bromide (CTAB) protocol of Lanes et al. (2013). Microsatellite analysis was performed on 9 microsatellite loci, 6 *A. aculeata* loci (Aacu07, Aacu10, Aacu12, Aacu26, Aacu30, and Aacu45) developed by Nucci et al. (2008) and 3 *E. guineensis* loci: P1T14 (Singh et al. 2007), mEgCIR3365 and mEgCIR0840 (Billotte et al. 2005).

Polymerase chain reaction (PCR) was carried out in a Veriti™ thermal cycler (Applied Biosystems). Each reaction had a total volume of 20 µL and contained 30 ng genomic DNA, 100 µM dNTPs, 0.1 µM SSR primer, 0.5 U Taq polymerase, and 1X PCR buffer. The thermocycler was programed for one initial predenaturation step of 5 min at 94 °C followed by 30 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, and with a final extension step of 8 min at 72 °C. The resulting DNA amplicons were separated by electrophoresis on a 6.0% (w/v) denaturing polyacrylamide gel (19:1 acrylamide:bis-acrylamide, 7.5 M urea, 5.0× TBE) using the SequiGen GT electrophoresis system (Bio-Rad). The gels were visualized by silver nitrate staining.

Genetic Diversity Between Developmental Stages of *A. aculeata*

To compare the genetic diversity between adult (n = 27) and juvenile (n = 157) plants, the total and average number of alleles per locus (k), the effective number of alleles per locus (N_e), the observed heterozygosity (H_o), and the unbiased expected heterozygosity (H_e) were estimated for each individual SSR locus tested, using the GenAlEx 6.5 software (Peakall and Smouse 2006). To compare the average values of k, H_o, and H_e between the life stages, their 95% confidence intervals (CIs) were calculated using the standard error (mean ± 1.96 × SE). The allelic richness (A_r), a measure of the number of alleles irrespective of sample size, was calculated using the rarefaction technique implemented in FSTAT 2.9.3.2 software (Goudet 1995). Inbreeding between adult and juvenile plants was estimated by the fixation index (F) and its significance (i.e., $F \neq 0$). The significance of the F values was tested with 10.000 permutations (alleles among individuals) using Bonferroni's correction (95%, α = 0.05). For juvenile plants, inbreeding was estimated by the intra-individual F, using as reference allelic frequencies those values determined for adult plants by the SPAGeDI 1.4 software (Hardy and Vekemans 2002).

Mating System Analysis

The mating system parameters of *A. aculeata* were calculated for each maternal family (n = 19, Figure 1) and at the population level based on mixed and correlated mating models (Ritland and Jain 1981; Ritland 1989) using the MLTR 3.4 software (Ritland 2002). The estimated parameters were: multilocus outcrossing rate (t_m), single-locus outcrossing rate (t_s), mating among relatives ($t_m - t_s$), correlation of selfing (r_s), multilocus paternity correlation ($r_{p(m)}$), and the inbreeding coefficient of maternal parents ($\hat{F}_m$). The 95% CI based on standard error was estimated by the bootstrap method based on 1000 replications. The expectation–maximization method (Ritland 2002) was used to solve the likelihood equation, which is recommended for data sets with missing data and when assuming the presence of undetected null alleles. Subsequently, based on the

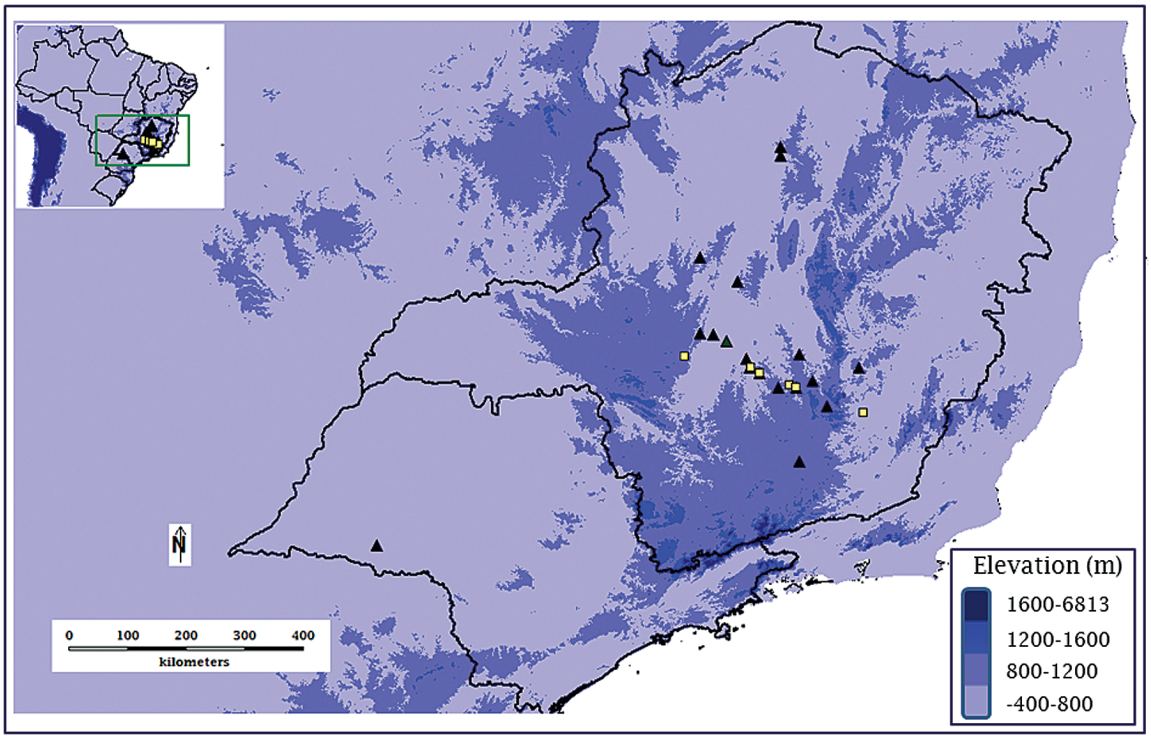

Figure 1. Geographical location in southeastern Brazil of: (▲) 19 Acrocomia *aculeata* mother trees and 157 of their progeny grown at the Macauba Active Germplasm Bank and used in the mating system study; and (□) 8 adult mother trees incorporated in the genetic diversity analysis. For more details of geographic locations and accessions codes, see Lanes et al. (2015).

determined mating system parameters, other genetic parameters were estimated as follows: the effective number of pollen donors contributing to each family ($N_{ep} = 1/r_{p(m)}$), following Ritland (1989). The average proportions of self-sibs ($\hat{P}_{ss} = \hat{s}^2$), half-sibs ($\hat{P}_{bs} = \hat{t}_m^2(1\hat{r}_{p(m)})$), full-sibs ($\hat{P}_{fs} = \hat{t}_m^2\hat{r}_{p(m)}$), and self-half-sibs ($\hat{P}_{sbs} = 2\hat{s}\hat{t}_m$), was estimated within families, where $\hat{s}^2 = (1 - \hat{t}_m)$ is the selfing rate (Feres et al. 2012). For these additional estimates, the 95% CIs were measured based on both upper and lower confidence limits calculated from the mating system parameters. The mathematical expressions used to determine the average proportions of relatedness within families, are conceptualized as:

(a) Self-sibs ($\hat{P}_{ss}$) = Descendants originating from selfing;
(b) Half-sibs ($\hat{P}_{bs}$) = Descendants have the mother in common, but fathers are different.
(c) Full-sibs ($\hat{P}_{fs}$) = Descendants have both parents (mother and father) in common.
(d) Self-half-sibs ($\hat{P}_{sbs}$) = Descendants have the mother in common; however some progenies originate from selfing, while others from outcrossing.

We also estimated the average coancestry coefficient ($\hat{\Theta}_{xy}$) among plants within progenies using the correlation coefficient of relatedness among plants within progenies ($\hat{r}_{xy}$). The coancestry coefficient for diploid species corresponds to half of the coefficient of relatedness within progenies ($\hat{\Theta}_{xy} = \hat{r}_{xy}/2$) (Ritland 1989)

$$\hat{r}_{xy} = 0.25(1 + \hat{F}_m)[4\hat{s} + (\hat{t}_m^2 + \hat{t}_m\hat{s}\hat{r}_s)(1 + \hat{r}_{p(m)})]$$

Next, the ancestry coefficient was used to estimate variance effective size, $\hat{N}_{e(v)} = 0.5/\hat{\Theta}_{xy}$ (Cockerham 1969). Although the minimum coancestry coefficient expected for half-sibs is 0.125, different levels of relatedness are expected within families (self-sibs, half-sibs, full-sibs, and self-half-sibs). Therefore, the $\hat{\Theta}_{xy}$ values may range between 0.125 and 1.0, which correspond to open-pollinated progenies. Those progenies often incorporate different kinds of relatives. In addition, the coefficient of coancestry (f_{ij}) for mother trees was calculated using Nason's estimator (Loiselle et al. 1995) using the SPAGeDi 1.4 software (Hardy and Vekemans 2002).

The coefficient of inbreeding in the progenies ($\hat{F}_o$) was inferred by calculating the fixation index using the GDA software (Lewis and Zaykin 2001). The fixation index and its CI were estimated by the bootstrap method based on 30 000 replications. The contributions of both self-fertilization and mating among relatives to inbreeding were also calculated, as $\hat{F}_s = 0.5\hat{s}(1 + \hat{F}_m)$ (Barrett and Kohn 1991) and $\hat{F}_{t_m - t_s} = \hat{F}_o - \hat{F}_s$ (Sebbenn 2006), respectively. Subsequently, the total coefficient of inbreeding in the progenies was estimated as $\hat{F}_o = \hat{F}_s + \hat{F}_{\hat{t}_m - \hat{t}_s}$, and Wright's equilibrium inbreeding coefficient was estimated as $\hat{F}_{eq} = (1 - t_m)/(1 + t_m)$, where t_m is the multilocus outcrossing rate (Fyfe and Bailey 1951). The number of mother tree seed donors necessary to retain a reference effective population size ($N_{e(\text{reference})}$) of 150 (Nunney and Campbell 1993), was estimated following the method of Sebbenn (2006) and based on the relationship between the effective population number goal of the breeding program ($N_{e(\text{reference})}$) and the effective population size of the average variance estimated for plants within progenies $\hat{N}_{e(v)}$: $\hat{m} = N_{e(reference)}/\hat{N}_{e(v)}$. It is noteworthy that this estimate was based on 2 assumptions about the mother trees: 1) that they are not related and do not mate with each other, and 2) that they receive pollen from a different gene pool, that is, there is no overlapping. It is important to note that the ($N_{e(\text{reference})}$) is related to genetic size representation rather than the physical size. Thus, this parameter will allow scale and specify the optimal sample size to be collected, in order to retain given level of genetic variability.

To find the allelic frequency (ωaf) retained in the reference population ($N_{e(\text{reference})}$), we followed Resende (2002)

$$\omega af = p_0 \pm z\{[p_0(1 - p_0)]/[2N_e]\}^{0.5}$$

where p_0 is the parametric frequency of the allele in the original population; Z is the tabulated value of the normal distribution, equal to 1.96 for a 95% CI; and N_e is value of the effective population size. If the CI lower limit is different from zero, it can be inferred that the allelic frequency (arbitrary test) was captured in the population.

In fulfillment of data archiving guidelines (Baker 2013), we have deposited the primary data underlying these analyses in Dryad.

Results

Genetic Diversity

Juvenile plants exhibited a total number of alleles higher than that of adult plants ($k = 55$ versus $k = 48$) for all 9 microsatellite loci tested, which corresponds to an average of 6.1 and 5.3 alleles, respectively. The A_r per locus ranged between 2 and 8 alleles for both life stages accessed, whereas the average effective number of alleles was of 3.8 and 3.4 for adult and juvenile plants, respectively. However, the observed mean heterozygosity in adult plants ($H_o = 0.527$) was higher than that observed in juveniles ($H_o = 0.441$). In juvenile plants, the P1T14 locus showed levels of observed heterozygosity (H_o) higher than expected (H_e), whereas in adult plants, both the P1T14 and mEgCIR3365 loci showed higher than expected levels of H_o. The analysis detected unique alleles ($k = 7$) only in juvenile trees. For juvenile plants, the fixation index (F) was positive and significantly different from zero for 8 of the 9 loci tested (Aacu07, Aacu45, Aacu12, Aacu26, Aacu10, Aacu30, mEgCIR3365, and mEgCIR0840) and for the overall juvenile plant average ($F_{of} = 0.433$). For adult trees, F was not significantly different from zero except for mEgCIR3365 and P1T14, for which it was negative and significant.

Outcrossing Rate and Mating System Parameters

The multilocus outcrossing rate for *A. aculeata* was high ($\hat{t}_m = 0.986$), and the population estimates did not differ statistically from unity (Table 2). The single-locus outcrossing rate was significantly different from unity ($\hat{t}_s = 0.603$, $P < 0.05$), as shown by the CI. The difference between the multilocus and single-locus outcrossing rates was high and significantly different from zero ($\hat{t}_m - \hat{t}_s = 0.383$), indicating the occurrence of mating among relatives. The correlation of selfing was significantly different from zero, yet low ($\hat{r}_s = 0.046$), indicating low individual variation in outcrossing rate; however, the estimated multilocus paternity correlation was significantly higher than zero ($\hat{r}_{p(m)} = 0.564$, $P < 0.05$) in the population. This finding is compatible with the restricted effective number of pollen-donor trees ($\hat{N}_{ep} = 1.773$). Estimates of the inbreeding coefficient of maternal parents ($\hat{F}_m = 0.309$) and of progeny ($\hat{F}_o = 0.154$) were high and positive, which indicates the occurrence of endogamy, although only the former was significantly different from zero ($P < 0.05$). The prominent fraction of endogamy observed in the progeny is due to mating among relatives ($\hat{F}_{t_m - t_s} = 0.145$). This fact is supported by the lower value of the inbreeding coefficient in Wright's equilibrium

Table 1. Estimates of genetic diversity and fixation index of *Acrocomia aculeata* adult and juvenile plants

	Adults (n = 27)						Juveniles (n = 157)					
Locus	k	A_r	N_e	H_o	H_e	F	k	A_r	N_e	H_o	H_e	F_{of}
Aacu07	8	7.9	4.7	0.577	0.801	0.285	8	8.0	4.1	0.532	0.757	0.327*
Aacu45	4	3.9	1.9	0.481	0.494	0.026	4	4.0	2.2	0.329	0.557	0.475*
Aacu12	6	6.0	3.5	0.360	0.725	0.514	7	7.0	3.4	0.278	0.709	0.651*
Aacu26	7	7.0	4.0	0.407	0.762	0.474	8	8.0	4.4	0.367	0.775	0.558*
Aacu10	7	6.9	3.9	0.593	0.760	0.224	7	7.0	5.1	0.631	0.808	0.278*
Aacu30	5	5.0	3.7	0.519	0.744	0.308	7	7.0	3.6	0.306	0.728	0.608*
mEgCIR3365	2	2.0	1.9	0.667	0.492	–0.362*	2	2.0	1.8	0.399	0.453	0.126*
mEgCIR0840	6	6.0	4.2	0.211	0.741	0.795	7	7.0	6.0	0.353	0.837	0.751*
P1T14	3	3.0	2.4	0.926	0.586	–0.590*	5	5.0	3.1	0.771	0.677	–0.049*
Mean	5.3	5.3	3.4	0.527	0.678	0.219	6.1	6.1	3.8	0.441	0.700	0.433*
$CI_{[Class\text{-}inf]}$	4.0	—	2.9	0.389	0.597	0.141	4.8	—	2.7	0.331	0.619	0.402
$CI_{[Class\text{-}Sup]}$	6.6	—	4.6	0.664	0.759	0.297	7.4	—	4.0	0.550	0.781	0.464
Total	48	—	—	—	—	—	55	—	—	—	—	—

k, number of alleles per locus; A_r, allelic richness; N_e, effective number of alleles per locus; H_o and H_e, observed and expected heterozygosity, respectively; F and F_{of}, fixation index for adult (mother trees) and juvenile (offspring) plants, respectively; F_{of}, intra-individual fixation index calculated with the reference allele frequencies obtained for the adult tree using the SPAGeDI program; CI, confidence interval (95%, α = 0.05).
*$P < 0.05$ following Bonferroni correction.

Table 2. Mating system parameters, estimates of inbreeding, and relatedness of *Acrocomia aculeata*

	Estimate	95% CI
Mating system		
Multilocus outcrossing rate: $\hat{t}_m$	0.986	0.961–1.000
Single-locus outcrossing rate: $\hat{t}_s$	0.603	0.534–0.672
Selfing rate: $\hat{s} = 1 - \hat{t}_m$	0.014	0.000–0.039
Mating among relatives: $\hat{t}_m - \hat{t}_s$	0.383	0.312–0.454
Correlation of selfing: $\hat{r}_s$	0.046	0.011–0.081
Multilocus paternity correlation: $\hat{r}_{p(m)}$	0.564	0.272–0.856
Effective number of pollen donors: $\hat{N}_{ep}$	1.773	1.168–3.677
Inbreeding and genetic structure		
Inbreeding coefficient of maternal parents: $\hat{F}_m$	0.309	0.240–0.378
Inbreeding coefficient of progeny: $\hat{F}_o$	0.154	0.000–0.298
Inbreeding in progeny from selfing: $\hat{F}_s$	0.009	0.000–0.024
Inbreeding in progeny from mating among relatives: $\hat{F}_{t_m - t_s}$	0.145	0.000–0.274
Wright's equilibrium inbreeding coefficient: $\hat{F}_{eq}$	0.007	0.000–0.020
Proportion (%) of self-sibs pairs: $\hat{P}_{ss}$	0.020	0.000–0.156
Proportion (%) of half-sibs pairs: $\hat{P}_{hs}$	42.388	14.396–67.169
Proportion (%) of full-sibs pairs: $\hat{P}_{fs}$	54.832	25.091–85.604
Proportion (%) of self-half-sibs pairs: $\hat{P}_{shs}$	2.761	0.000–7.584
Coancestry within offspring: $\hat{\Theta}_{xy}$	0.256	0.218–0.304
Nason's estimator of kinship coefficient: $\hat{f}_{ij}$	0.229	0.136–0.324
Variance effective size: $\hat{N}_{e(v)}$	1.937	1.646–2.294
Number of seed-trees for seed collection: $\hat{m}$	77	65–91
Allelic frequency retained: ω_{af}	0.02	0.004–0.036[a]
Sample size		
Number of mothers trees	19	
Average number of offspring for mothers trees	8.3	

CI, confidence interval calculated by 1000 bootstraps.
[a]Confidence interval calculated for the allelic frequency of 2% (arbitrary test).

($\hat{F}_{eq}$ = 0.007) in comparison to the inbreeding coefficient of progeny ($\hat{F}_o$ = 0.154). The Nason's estimator of coancestry coefficient for maternal trees was $\hat{f}_{ij}$ = 0.229, and the 95% CI after 10 000 permutations ranged from 0.136 to 0.324. The mean coancestry coefficient within progeny ($\hat{\Theta}_{xy}$ = 0.258) was greater than expected for half-sib families (0.125), suggesting that families comprise a variety of different types of relatedness. The progenies of *A. aculeata* were predominantly composed of full-sibs (54.83%) and half-sibs (42.39%). The variance effective size ($N_{e(v)}$ = 1.9) was lower than expected for panmictic populations ($N_{e(v)}$ = 4). In order to have an effective population size of 150 and also capture of alleles (ω_{af}) with a frequency greater or equal to 2% in the original population, the required number of mother trees ($\hat{m}$) for seed collection was estimated to be at least 77 palm trees (Table 2).

The multilocus outcrossing rates ($\hat{t}_m$) among families were variable, ranging from 0.835 to 1.000. Estimates for $\hat{t}_m$ were significantly different from unity ($P < 0.05$) in 15 out of the 19 assessed families. There were notable differences among families in regards to: 1) mating among relatives ($\hat{t}_m - \hat{t}_s$ = 0.064–0.538), 2) the multilocus paternity correlation rates ($\hat{r}_{p(m)}$ = 0.035–0.998), and 3) the effective number of pollen-donor trees ($\hat{N}_{ep}$ = 1.0–28.6).

Discussion

In this study, we asked whether the levels of heterozygosity differ between the juvenile and adult stages of *A. aculeata*. We also asked whether the mating system of this species can be characterized as being predominantly a mixed mating or an outcrossing system. We found that observed heterozygosity levels varied between the juvenile and adult stages and were higher in the latter, indicating that selection against homozygosis takes place during the course of the plant life cycle. The *A. aculeata* mating system is predominantly outcrossing, which confers high genetic variability on the species. Estimates for its mating system parameters suggest that there is individual variation for self-incompatibility, which can be a survival strategy adopted in isolated or fragmented habitats.

Table 3. Mating system and within-family genetic parameters for *Acrocomia aculeata*

Family / [*n*]	$\hat{t}_m$ (SE)	$\hat{t}_m - \hat{t}_s$ (SE)	$\hat{r}_{p(m)}$ (SE)	$\hat{N}_{ep}$	$\hat{\Theta}_{xy}$	$\hat{N}_{e(v)}$	$\hat{m}$
BGP11 [09]	0.979 (0.009)*	0.200 (0.059)*	0.086 (0.022)*	11.6	0.184	2.71	55
BGP13 [09]	0.996 (0.000)*	0.246 (0.054)*	0.690 (0.115)*	1.4	0.277	1.80	83
BGP15 [10]	1.000 (0.000)	0.233 (0.044)*	0.880 (0.095)	1.1	0.308	1.63	92
BGP16 [09]	0.998 (0.002)	0.300 (0.040)*	0.227 (0.038)*	4.4	0.201	2.48	60
BGP27 [10]	0.990 (0.004)*	0.309 (0.047)*	0.071 (0.037)	14.1	0.178	2.80	54
BGP29 [08]	0.900 (0.000)*	0.162 (0.059)*	0.100 (0.001)*	10.0	0.213	2.35	64
BGP31 [09]	1.000 (0.000)	0.538 (0.042)*	0.998 (0.012)	1.0	0.327	1.53	98
BGP33 [08]	0.900 (0.000)*	0.064 (0.026)*	0.100 (0.001)*	10.0	0.213	2.35	64
BGP36 [10]	0.835 (0.001)*	0.198 (0.035)*	0.173 (0.095)	5.8	0.243	2.06	73
BGP37 [10]	1.000 (0.003)	0.331 (0.036)*	0.675 (0.158)*	1.5	0.274	1.82	82
BGP40 [09]	0.999 (0.000)*	0.192 (0.038)*	0.184 (0.062)*	5.4	0.194	2.58	58
BGP47 [06]	0.990 (0.004)*	0.463 (0.038)*	0.705 (0.117)*	1.4	0.280	1.78	84
BGP48 [04]	0.990 (0.004)*	0.246 (0.039)*	0.682 (0.121)*	1.5	0.277	1.81	83
BGP50 [10]	0.990 (0.004)*	0.503 (0.041)*	0.512 (0.146)*	2.0	0.249	2.01	75
BGP63 [04]	0.990 (0.004)*	0.210 (0.050)*	0.035 (0.013)*	28.6	0.173	2.90	52
BGP65 [08]	0.953 (0.016)*	0.409 (0.059)*	0.100 (0.001)*	10.0	0.195	2.56	59
BGP74 [10]	0.953 (0.016)*	0.225 (0.038)*	0.100 (0.001)*	10.0	0.195	2.56	59
BGP78 [08]	0.979 (0.009)*	0.427 (0.032)*	0.381 (0.161)*	2.6	0.231	2.17	69
BGP87 [06]	0.979 (0.009)*	0.507 (0.031)*	0.302 (0.128)*	3.3	0.218	2.29	66

n, number of progeny per family; $\hat{t}_m$, multilocus outcrossing rate; $\hat{t}_m - \hat{t}_s$, outcrossing rate among relatives; $\hat{r}_{p(m)}$, multilocus paternity correlation; $\hat{N}_{ep}$, effective number of pollen donors; $\hat{\Theta}_{xy}$, coefficient of coancestry within families; $\hat{N}_{e(v)}$, variance effective size within family; $\hat{m}$, number of mother trees necessary for seed collection. The selfing correlation coefficient, $\hat{r}_s$, ranged from 0.029 to 0.117. Numbers in brackets refer to the standard error calculated from 1000 bootstraps.

*$P < 0.05$.

Genetic Diversity

Our results reaffirm the great genetic variability of *A. aculeata* present in southeastern Brazil, as reported by Lanes et al. (2015). Furthermore, the present study reveals that the 2 life stages, that is, the juvenile and adult plants, exhibited very similar and high levels of genetic diversity (allelic richness and effective number of alleles), despite the widespread heterozygous deficiency for all SSR loci tested (F and F_{of}). The unique alleles ($k = 7$) found exclusively in the juvenile plants are likely the result of unsampled adult individuals in the population, new allelic forms introduced by neighboring populations, or even by gene flow from other localities. Similar observations were also reported for *B. eriospatha* (Areacaceae) by Nazareno and Reis (2014).

The low observed heterozygosity values and the high positive estimates of the fixation index attained by the juvenile plants in comparison to adult plants suggest a higher degree of endogamy in the younger stage and, consequently, selection against homozygotes. When comparing the initial phases of the plant life cycle (seed, seedling, and juvenile) with the adult stage, selection against homozygotes is also observed in other plant species (Eguiarte et al. 1992; Gaiotto et al. 2003; Carneiro et al. 2011). The high level of heterozygosity for the P1T14 locus in both juvenile and adult *A. aculeata* plants suggests that this locus is inserted into a genic region, or even linked to a gene, that is under selection pressure, a possibility also advanced for a single microsatellite locus in *Theobroma cacao* (Silva et al. 2011).

The excess homozygosis indicated by the fixation index in the juvenile stage of *A. aculeata* suggests that inbreeding is recurrent, probably due to mating among relatives and/or by selfing. This information is relevant, because the inbreeding depression is caused by increased homozygosity, which, in turn, has a strong negative effect on survival and reproduction of the progeny, contributing to their gradual loss throughout the plant life cycle.

Mating System of *A. aculeata*

The mating system parameters that were determined on the basis of mixed and correlated mating models (Ritland and Jain 1981; Ritland 1989) reveal high levels of outcrossing ($\hat{t}_m > 0.98$) at the population level, with a selfing rate of $\hat{s} < 5\%$ based on a 95% CI (Table 2). Furthermore, high estimates of outcrossing ($\hat{t}_m > 95\%$) were also observed in 16 families (84%). These results indicate that *A. aculeata* is predominantly outcrossing (allogamous) according to the selfing rate for hermaphrodite plants species ($\hat{s} < 20\%$) classified by Schemske and Lande's (1985), and accepted by many authors (Goodwillie et al. 2005; Escobar et al. 2011; Winn et al. 2011). Our finding contrasts with previous studies showing a greater selfing rate ($20 < \hat{s} < 80\%$) for *A. aculeata*, which would characterize this species as having a mixed mating system (Scariot et al. 1991; Nucci et al. 2008; Abreu et al. 2012).

Acrocomia aculeata showed a low number of progenies by selfing ($\hat{s} = 0.014$). One of the possible causes for this result is the existence of an excessive mutational load. This condition is strongly associated with high rates of early fruit abortion and elimination of endogamic individuals in their early phase of development, when environmental stressors are a real danger (Husband and Schemske 1996; Wilcock and Neiland 2002). Inbreeding depression in the early stages of the life cycle is characteristic of allogamous plants, and it is associated with the presence of highly deleterious recessive alleles (Husband and Schemske 1996; Mustajärvi et al. 2005; Thiele et al. 2010). In addition to common postzygotic risk factors due to inbreeding (fruit abortion, low viability of the seed, and seedling mortality), it is likely that *A. aculeata* has developed another type of self-incompatibility system (Abreu et al. 2012). As an exemple, the occurrence of late-acting self-incompatibility (LSI), poorly understood phenomenon, but very common in woody cerrado species (Oliveira and Gibbs 2000). This hypothesis is further strengthened if we apply the "index of self-genetic incompatibility" (ISI) proposed by Zapata and Arroyo

(1978) and Bullock (1985) to the percentage of *A. aculeata* fruit obtained from controlled pollination in a previous study (Scariot et al. 1991). The obtained value (ISI = 0.26) suggests that this species is partially self-incompatible.

Nevertheless, there is a chance that some families produced more progenies by selfing than others, as indicated by differences in the selfing correlation coefficient among families ($0.029 \leq \hat{r}_s \leq 0.117$, Table 3), possibly because of variation in the self-incompatibility system among trees within populations or certain groups. According to Del Castillo and Trujillo (2008), the true range of variation of mating systems within species is difficult to ascertain.

Mating among Relatives and Correlated Mating

The high and significant levels of biparental inbreeding ($\hat{t}_m - \hat{t}_s = 0.383$) portray a high degree of relatedness between parents involved in the reproductive process, which can have a strong impact on population viability as a result of the presence of recessive deleterious alleles fixed in the homozygous state (Keller and Waller 2002). In addition, the high multilocus paternity correlation rate ($\hat{r}_{p(m)} = 0.564$) indicates that a large proportion of the progeny were generated by the same maternal and paternal plant (biparental outcrossing), suggesting that cross-pollination is not a random event.

Correlated matings imply that a small number of trees contributed to the effective pollination. This constraint on the number of paternal contributors was confirmed by the extremely low effective number of pollen donors ($\hat{N}_{ep}$), which presented an average of 2 individuals per tree (Table 2). A similar finding was reported for the palm *B. eriospatha* (Nazareno and Reis 2012). These results are possibly linked to the existence of intrapopulation spatial genetic structure, since *A. aculeata* has a gregarious habit and is often found in large groves in the wild. Species that form these large population clusters usually have a high degree of relatedness (Smouse and Sork 2004). Thus, the spatial genetic structure favors biparental inbreeding in the offspring, which in this study was confirmed by the estimates of inbreeding in the progeny from mating among relatives ($\hat{F}_{t_m - t_s}$). In fact, for *A. aculeata*, mating among relatives was the main cause of inbreeding, when compared with the endogamous component of selfing ($\hat{F}_s$).

One characteristic that favors the formation of intrapopulation spatial genetic structure in *A. aculeata*, and consequently the high level of endogamy in its progeny, is that fruit dispersion is primarily barochoric, which limits the distance that fruit travels from the mother plant (Abreu et al. 2012; Lanes et al. 2015). This type of limitation is also observed in other palms, such as *Attalea phalerata* and *Astrocaryum mexicanum*, where most seeds are found within 30 m from the mother tree (Eguiarte et al. 1993; Choo et al. 2012). Although zoochory also occurs in *A. aculeata*, this process depends on synchronized occurrence of vertebrates and fruits (Pott and Pott 1994; Scariot 1998; Eiserhardt et al. 2011).

Another cause of inbreeding in *A. aculeata* would be the association between its androgynous inflorescence and the behavior of pollinators systematically visiting the same inflorescence or inflorescences of neighboring trees. The mean distance traveled by the pollen dispersed by entomophily or anemophily in some palm trees species is relatively short, 303 m for *O. bataua* (Ottewell et al. 2012), 71 m for *Phoenix canariensis* (Saro et al. 2014), 200 m for *Iriartea deltoidea* (Sezen et al. 2007), and 20 m for *Astrocaryum mexicanum* (Eguiarte et al. 1993). For *A. aculeata,* beetles are the main pollinators (Scariot et al. 1991), and their limited flight range would restrict gene flow (Lanes et al. 2015).

In this study, 3 maternal families of *A. aculeata* (BGP29, BGP33, and BGP36) showed relatively high rates of selfing ($\hat{s}$ = 10–16.5%), possibly because their mother trees were isolated due to anthropization pressure upon the original habitats, which are located in the vicinity of densely populated urban areas (IBGE 2010). The fragmentation of tropical forests has resulted in a decrease in the number of pollinators and floral visitors (Bergsdorf 2006; Nayak and Davidar 2010), reducing gene flow and causing loss of genetic diversity. In addition to favoring inbreeding, habitat fragmentation increases the rate of inflorescence abortion and nonviable seeds in various species of palms, including *Astrocaryum mexicanum, Oenocarpus bacaba, Mauritia flexuosa*, and *B. eriospatha* (Lepsch-Cunha et al. 2003; Aguirre and Dirzo 2008; Federman et al. 2014; Nazareno and Reis 2014). Moreover, seed dispersers such as primates, small marsupials, and birds are also sensitive to habitat fragmentation (Laurance et al. 2002). According to Portela and Santos (2014), such fragmentation has led to a lack of seed-dispersing animals for the palm *Astrocaryum aculeatissimum*, which has contributed to the reduction of populations of this species in southeastern Brazil.

The estimate of coancestry within the progenies of *A. aculeata* (Table 2) indicates a probability of 25.6% that 2 alleles sampled in 2 plants of the same progeny are identical by descent. This result suggests that the analyzed progenies comprise mixtures of different kinds of relatedness. In fact, this mix of relatedness between the progenies is supported by the variance effective size ($\hat{N}_{e(v)} = 1.9$), indicating that the largest fraction of the progenies are full-sibs. The genetic representativeness in the progenies of *A. aculeata* is 52% lower than that expected in random mating progenies for an idealized perfectly panmictic population where the variance effective size equals 4.0 ($\hat{N}_{e(v)} = 0.5 / \hat{\Theta}_{xy} = 0.5 / 0.125$) and corresponds to half-sib progenies. Thus, in *A. aculeata*, mating among relatives and correlated matings were responsible for deviations from the assumptions that characterize an idealized population, which caused a decrease in the variance effective size. These deviations imply the need to collect larger samples to guarantee the maintenance of genetic variability in *in situ* and *ex situ* genetic conservation programs. According to Namoff et al. (2010), the *ex situ* genetic conservation of a total of 15 individuals (seeds) collected from 3 mother trees (half-sib families) of *Leucothrinax morrisii* palm ensures the capture of more than 80% of the allelic diversity of the original population. However, in families with many full-sibs a greater number of mother-trees will be necessary.

Implications for Breeding and Genetic Conservation

Many breeding programs assume that the progenies of allogamous species, like *A. aculeata,* originate from random mating. However, the mating system analysis for *A. aculeata* reveals deviations from the assumption of panmixia, indicating that most of the progenies are full-sibs. This information has important implications for breeding, because if open-pollinated progenies of *A. aculeata* are assumed to be half-sibs, the additive genetic variance, heritability coefficient, and genetic gains from selection would be overestimated. For instance, if open-pollinated progenies are collected from nonrelated populations with no inbreeding, genetic correlation among plants within families, that is, the coefficient of relatedness ($\hat{r}_{xy}$), would be 1/4 (0.25) of additive genetic variance (Namkoong 1966). Since $\hat{r}_{xy}$ among plants within the progenies doubles the coancestry coefficient ($\hat{r}_{xy} = 2\hat{\Theta}_{xy}$) in diploid species (Lynch and Walsh 1998), the actual estimate of the coefficient of relatedness of *A. aculeata* would equal 0.512 of additive genetic variance ($0.512\sigma^2_A$).

Deviations from the assumptions that characterize an idealized population also have implications for the *ex situ* genetic conservation and seed collection of *A. aculeata*. To retain an effective population size of 150, it will be necessary to collect seeds from at least 77 mother trees of the population located in the studied area (southeastern Brazil). This effective population size ensures capture of alleles (ω_{af}) with a frequency greater or equal to 2% in the original population. This value is below the required minimum ω_{af} (≥4%) for breeding programs, according to Resende (2002). However, according to this same author, if the goal is the use of the species for *in situ* conservation and recovery of degraded areas, the number of *A. aculeata* trees should be raised to 258 individuals in order to maintain an effective population size of 500 and to retain alleles with a frequency as low as 0.4% in the original population.

This analysis of the mating system and genetic diversity of *A. aculeata* representatives from natural populations in southeastern Brazil showed that the species has a predominantly outcrossing mating system as well as higher levels of heterozygosity in adults than in juveniles. Additionally, we estimated mating system parameters that can be used to support decision making in conservation and breeding programs, allowing the development of more precise mathematical models and estimation of the appropriate number of mother trees for seed collection. To our knowledge, this is the most comprehensive work on the mating system of *A. aculeata*, and the first to report differences in heterozygosity between its life stages. We hope that the information this report provides on the ecology and reproduction dynamics of *A. aculeata* will contribute to the domestication of this palm species as a new oil crop and source of renewable energy. And above all, once the process of domestication is completed, the palm can confirm its role as a *genetic resource model*, allowing a fully sustainable supply chain, with the potential to attend the major challenges of the today's world.

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Data Availability

Data deposited at Dryad: http://dx.doi.org/10.5061/dryad.5h50p

References

Abreu IS, Carvalho CR, Carvalho GMA, Motoike SY. 2011. First karyotype, DNA C-value and AT/GC base composition of macaw palm (*Acrocomia aculeata*, Arecaceae)—a promising plant for biodiesel production. *Aust J Bot*. 59:149–155.

Abreu AG, Priolli RH, Azevedo-Filho JA, Nucci SM, Zucchi MI, Coelho RM, Colombo CA. 2012. The genetic structure and mating system of Acrocomia aculeata (Arecaceae). *Genet Mol Biol*. 35:119–121.

Aguirre A, Dirzo R. 2008. Effects of fragmentation on pollinator abundance and fruit set of an abundant understory palm in a Mexican tropical forest. *Biol Cons*. 141:375–384.

Baker CS. 2013. Journal of heredity adopts joint data archiving policy. *J Hered*. 104:1.

Barfod AS, Hagen M, Borchsenius F. 2011. Twenty-five years of progress in understanding pollination mechanisms in palms (Arecaceae). *Ann Bot*. 108:1503–1516.

Barrett SCH, Kohn J. 1991. The genetic and evolutionary consequences of small population size in plant: implications for conservation. In: Falk D, Holsinger KE, editors. *Genetics and conservation of rare plants*. New York: Oxford University Press. p. 3–30.

Bawa KS. 1974. Breeding systems of tree species of a lowland tropical community. *Evolution*. 28:85–92.

Bergsdorf T. 2006. Forest Fragmentation and Plant-Pollinator Interactions in Western Kenya [Dissertation Doktorgrades]. [Bonn (Germany)]: Rheinische Friedrich-Wilhelms-Universität.

Berton LHC, Azevedo Filho JA, Siqueira WJ, Colombo CA. 2014. Seed germination and estimates of genetic parameters of promising macaw palm (*Acrocomia aculeata*) progenies for biofuel production. *Ind Crop Prod*. 51:258–266.

Billotte N, Marseillac N, Risterucci A-M, Adon B, Brottier P, Baurens F-C, Singh R, Herrán A, Asmady H, Billot C, *et al.* 2005. Microsatellite-based high density linkage map in oil palm (*Elaeis guineensis* Jacq.). *Theor Appl Genet*. 110:754–765.

Bora PS, Rocha RVM. 2004. Macaíba palm: fatty and amino acids composition of fruits. *Cienc Tecnol Aliment*. 4:158–162.

Bullock SH. 1985. Breeding systems in the flora of a tropical deciduous forest in Mexico. *Biotropica*. 17:287–301.

Carneiro FS, Lacerda AEB, Leme MR, Gribel R, Kanashiro M, Wadt LHO, Sebbenn AM. 2011. Effects of selective logging on the mating system and pollen dispersal of *Hymenaea courbaril* L. (leguminosae) in the Eastern Amazon as revealed by microsatellite analysis. *Forest Ecol Manag*. 262:1758–1765.

Choo J, Juenger TE, Simpson BB. 2012. Consequences of frugivore-mediated seed dispersal for the spatial and genetic structures of a neotropical palm. *Mol Ecol*. 21:1019–1031.

Ciconini G, Favaro SP, Roscoe R, Miranda CHB, Tapeti CF, Miyahira MAM, Bearari L, Galvani F, Borsato AV, Colnago LA, *et al.* 2013. Biometry and oil contents of *Acrocomia aculeata* fruits from the Cerrados and Pantanal biomes in Mato Grosso do Sul, Brazil. *Ind Crop Prod*. 45:208–214.

Coimbra MC, Jorge N. 2012. Fatty acids and bioactive compounds of the pulps and kernels of Brazilian palm species, guariroba (*Syagrus oleraces*), jerivá (*Syagrus romanzoffiana*) and macaúba (*Acrocomia aculeata*). *J Sci Food Agric*. 92:679–684.

Cockerham CC. 1969. Variance of gene frequencies. *Evolution*. 23:72–84.

Del Castillo RF, Trujillo S. 2008. Effect of inbreeding depression on outcrossing rates among populations of a tropical pine. *New Phytol*. 177:517–524.

Dudash MR, Fenster CB. 2000. Inbreeding and outbreeding depression in fragmented populations. In: Young AC, Clarke GM, editors. *Genetics, demography and viability of fragmented populations*. Cambridge (UK): Cambridge University Press. p. 35–54.

Eguiarte LE, Burquez A, Rodriguez J, Martinez-Ramos M, Sarukhan J, Pinero D. 1993. Direct and indirect estimates of neighborhood and effective population size in a tropical palm, *Astrocaryum mexicanum*. *Evolution*. 47:75–87.

Eguiarte LE, Perez-Nasser N, Piñero D. 1992. Genetic structure, outcrossing rate and heterosis in *Astrocaryum mexicanum* (tropical palm): implications for evolution and conservation. *Heredity*. 69:217–228.

Eiserhardt WL, Svenning JC, Kissling WD, Balslev H. 2011. Geographical ecology of the palms (Arecaceae): determinants of diversity and distributions across spatial scales. *Ann Bot*. 108:1391–1416.

Escobar JS, Auld JR, Correa AC, Alonso JM, Bony YK, Coutellec MA, Koene JM, Pointier JP, Jarne P, David P. 2011. Patterns of mating-system evolution in hermaphroditic animals: correlations among selfing rate, inbreeding depression, and the timing of reproduction. *Evolution*. 65(5):1233–1253.

Falconer DS. 1989. *Introduction to quantitative genetics*. 3rd edn. New York: Longman.

Federman S, Hyseni C, Clement W, Oatham MP, Caccone A. 2014. Habitat fragmentation and the genetic structure of the Amazonian palm *Mauritia flexuosa* L.f. (Arecaceae) on the island of Trinidad. *Conserv Genet*. 15:355–362.

Feres JM, Sebbenn AM, Guidugli MC, Mestriner MA, Moraes MLT, Alzate-Marin AL. 2012. Mating system parameters at hierarchical levels of fruits, individuals and populations in the Brazilian insect-pollinated tropical tree, *Tabebuia roseo-alba* (Bignoniaceae). *Conserv Genet*. 13:393–405.

Fuchs E, Hamrick JL. 2011. Mating system and pollen flow between remnant populations of the endangered tropical tree, *Guaiacum sanctum* (Zygophyllaceae). *Conserv Genet*. 12:175–185.

Fyfe JL, Bailey NTJ. 1951. Plant breeding studies in leguminous forage crops. 1. Natural cross breeding in winter beans. *J Agr Sci*. 41:371–378.

Gaiotto FA, Grattapaglia D, Vencovsky R. 2003. Genetic structure, mating system, and long-distance gene flow in heart of palm (*Euterpe edulis* Mart.). *J Hered*. 94:399–406.

Galetti M, Zipparro VB, Morellato LPC. 1999. Fruiting phenology and frugivory on the palm *Euterpe edulis* in a lowland Atlantic forest of Brazil. *Ecotropica*. 5:115–122.

Genini J, Galettia M, Morellato LPC. 2009. Fruiting phenology of palms and trees in an Atlantic rainforest land-bridge island. *Flora* 204:131–145.

Gonçalves DB, Batista AF, Rodrigues MQ, Nogueira KM, Santos VL. 2013. Ethanol production from macaúba (*Acrocomia aculeata*) presscake hemicellulosic hydrolysate by *Candida boidinii* UFMG14. *Bioresour Technol*. 146:261–266.

Goodwillie C, Kalisz S, Eckert CG. 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annu Rev Ecol Evol Syst*. 36:47–79.

Goudet J. 1995. FSTAT: a computer program to calculate F-statistics. *J Hered*. 86:485–486.

Hardy OJ, Vekemans X. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Mol Ecol Notes*. 2:618–620.

Henderson A. 1986. A review of pollination studies in the Palmae. *Bot Rev*. 52:221–259.

Henderson A, Galeano G, Bernal R. 1995. *Field guide to the palms of the Americas*. Princeton (NJ): Princeton University Press.

Hiane PA, Ramos Filho MM, Ramos MIL, Macedo MLR. 2005. Bocaiuva, *Acrocomia aculeata* (Jacq.) Lodd., pulp and kernel oils: characterization and fatty acid composition. *Braz J Food Technol*. 8:256–259.

Husband B, Schemske D. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution*. 50:50–74.

Instituto Brasileiro de Geografia e Estatística—IBGE. *Censo Demográfico 2010*. *Disponível em*: http://www.censo2010.ibge.gov.br

Lanes ECM, Nick C, Kuki KN, Freitas RD, Motoike SY. 2013. Genomic DNA isolation of *Acrocomia aculeata* (Arecaceae) from leaf and stipe tissue samples for PCR analysis. *Genet Mol Res*. 12:3905–3911.

Lanes ECM, Costa PMA, Motoike SY. 2014. Alternative fuels: Brazil promotes aviation biofuels. *Nature*. 511:31–31.

Lanes ECM, Motoike SY, Kuki KN, Nick C, Freitas RD. 2015. Molecular characterization and population structure of *Acrocomia aculeata* (Arecaceae) *ex situ* germplasm collection using microsatellites markers. *J Hered*. 106:102–112.

Laurance WF, Lovejoy TE, Vasconcelos HL, Bruna EM, Didham RK, Stouffer PC, Gascon C, Bierregaard RO, Laurance SG, Sampaio E. 2002. Ecosystem decay of Amazonian forest fragments: a 22-year investigation. *Conserv Biol*. 16:605–618.

Lepsch-Cunha N, Lund CA, Hamilton MB. 2003. Isolation and characterization of nuclear microsatellite loci in the tropical arboreal palm *Oenocarpus bacaba* (Arecaceae). *Mol Ecol Notes*. 3:435–437.

Lewis PO, Zaykin D. 2001. *Genetic data analysis: computer program for the analysis of allelic data*. Version 1.1 (d16c). Free program distributed by the authors. Available from http://phylogeny.uconn.edu/software.

Loiselle BA, Sork VL, Nason J, Graham C. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *Am J Bot*. 82:1420–1425.

Lynch M, Walsh B. 1998. *Genetics and analysis of quantitative traits*. Sunderland (MA): Sinauer Associates.

Keller L, Waller D. 2002. Inbreeding effects in wild populations. *Trends Ecol Evol*. 17:230–241.

Khanduri VP, Sharma CM, Kumar KS, Ghildiyal SK. 2013. Annual variation in flowering phenology, pollination, mating system, and pollen yield in two natural populations of *Schima wallichii* (DC.) Korth. *ScientificWorldJournal*. 2013:350157.

Markert JA, Champlin DM, Gutjahr-Gobell R, Grear JS, Kuhn A, McGreevy TJ Jr, Roth A, Bagley MJ, Nacci DE. 2010. Population genetic diversity and fitness in multiple environments. *BMC Evol Biol*. 10:205.

Motoike SY, Kuki KN. 2009. The potential of macaw palm (*Acrocomia aculeata*) as source of biodiesel in Brazil. *IRECHE*. 1:632–635.

Motoike SY, Lopes FA, Sá Júnior AQ, Carvalho M, Oliveira MAR. 2007. Processo de germinação e produção de sementes pré-germinadas de palmeiras do gênero *Acrocomia*. (Protocolo INPI 014070005335).

Murawski DA, Hamrick JL. 1991. The effect of the density of flowering individuals on the mating systems of nine tropical tree species. *Heredity*. 67:167–174.

Mustajärvi K, Siikamäki P, Akerberg A. 2005. Inbreeding depression in perennial *Lychnis viscaria* (Caryophyllaceae): effects of population mating history and nutrient availability. *Am J Bot*. 92:1853–1861.

Namkoong G. 1966. Inbreed effects on estimation of genetic additive variance. *For Sci*. 12:8–13.

Namoff S, Husby CE, Francisco-Ortega J, Noblick LR, Lewis CE, Griffith MP. 2010. How well does a botanical garden collection of a rare palm capture the genetic variation in a wild population? *Biol Cons*. 143:1110–1117.

Nayak KG, Davidar P. 2010. Pollinator limitation and the effect of breeding systems on plant reproduction in forest fragments. *Acta Oecol*. 36:191–196.

Nazareno AG, Reis MS. 2014. At risk of population decline? An ecological and genetic approach to the threatened palm species *Butia eriospatha* (Arecaceae) of Southern Brazil. *J Hered*. 105:120–129.

Nazareno AG, Reis MS. 2012. Linking phenology to mating system: exploring the reproductive biology of the threatened palm species *Butia eriospatha*. *J Hered*. 103:842–852.

Nucci SM, Azevedo-Filho JA, Colombo CA, Priolli RH, Coelho RM, Mata TL, Zucchi MI. 2008. Development and characterization of microsatellites markers from the macaw. *Mol Ecol Resour*. 8:224–226.

Núñez-Avellaneda LR, Bernal R, Knudsen JT. 2005. Diurnal palm pollination by mytropine beetles: is it weather-related? *Plant Syst Evol*. 254:149–171.

Nunney L, Campbell KA. 1993. Assessing minimum viable population size: demography meets population genetics. *Tree*. 8:234–239.

Oliveira PE, Gibbs PE. 2000. Reproductive biology of woody plants in a cerrado community of Central Brazil. *Flora*. 195:311–329.

Ottewell K, Grey E, Castillo F, Karubian J. 2012. The pollen dispersal kernel and mating system of an insect-pollinated tropical palm, *Oenocarpus bataua*. *Heredity (Edinb)*. 109:332–339.

Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel Population genetic software for teaching and research. *Mol Ecol Notes*. 6:288–295.

Pires TP, Souza ES, Kuki KN, Motoike SY. 2013. Ecophysiological traits of the macaw palm: a contribution towards the domestication of a novel oil crop. *Ind Crop Prod*. 44:200–210.

Portela RCQ, Santos FAM. 2014. Impact of forest fragment size on the population structure of three palm species (Arecaceae) in the Brazilian Atlantic Rainforest. *Rev Biol Trop*. 64:433–442.

Pott A, Pott VJ. 1994. *Plantas do pantanal*. Brasília (DF): EMBRAPA-SPI. p. 320.

Ramos SLF, Lopes MTG, Lopes R, Cunha RNV, Macêdo JLV, Contim LAS, Clement CR, Rodrigues DP, Bernardes LG. 2011. Determination of the mating system of Tucumã palm using microsatellite markers. *Crop Breed Appl Biotechnol*. 11:181–185.

Resende MDV. 2002. *Genética Biométrica e Estatística no Melhoramento de Plantas Perenes*. 1st edn. Embrapa Informação Tecnológica. p. 975.

Ritland K. 1989. Correlated matings in the partial selfer *Mimulus guttatus*. *Evolution*. 43:848–859.

Ritland K. 2002. Extensions of models for the estimation of mating systems using independent loci. *Heredity*. 88:221–228.

Ritland K, Jain S. 1981. A model for the estimation of outcrossing rate and gene frequencies using independente loci. *Heredity*. 47:35–52.

Salm R, Jalles-Filho E, Schuck-Paim C. 2005. A model for the importance of large arborescent palms in the dynamics of seasonally-dry Amazon forest. *Biota Neotrop*. 5:1–6.

Saro I, Robledo-Arnuncio JJ, González-Pérez MA, Sosa PA. 2014. Patterns of pollen dispersal in a small population of the Canarian endemic palm (*Phoenix canariensis*). *Heredity (Edinb)*. 113:215–223.

Sebbenn AM. 2006. Sistema de reprodução em espécies arbóreas tropicais e suas implicaçõess para a seleção de árvores matrizes para reflorestamentos ambientais. In: Higa AR, Silva LD, editors. *Pomares de sementes de espécies florestais nativas*. Curitiba: FUPEF. p. 193–198.

Scariot A. 1998. Seed dispersal and predation of the palm *Acrocomia aculeata*. *Principes*. 42:5–8.

Scariot A, Lleras E, Hay JD. 1991. Reproductive biology of the palm *Acrocomia aculeata* in Central Brazil. *Biotropica*. 23:12–22.

Schemske DW, Lande R. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution*. 39:41–52.

Sezen UU, Chazdon RL, Holsinger KE. 2007. Multigenerational genetic analysis of tropical secondary regeneration in a canopy palm. *Ecology*. 88:3065–3075.

Silva CR, Albuquerque PS, Ervedosa FR, Mota JW, Figueira A, Sebbenn AM. 2011. Understanding the genetic diversity, spatial genetic structure and mating system at the hierarchical levels of fruits and individuals of a continuous *Theobroma cacao* population from the Brazilian Amazon. *Heredity (Edinb)*. 106:973–985.

Silva JC, Barrichelo LEG, Brito JO. 1986. Endocarpos de babaçu e macaúba comparados a madeira de *Eucalyptus grandis* para a producão de carvão vegetal. *IPEF*. 34:31–34.

Singh R, Nagappan J, Tan SG, Panandam JM, Cheah SC. 2007. Development of simple sequence repeat (SSR) markers for oil palm and their application in genetic mapping and fingerprinting of tissue culture clones. *Asia Pac J Mol Biol Biotechnol*. 15:121–131.

Smouse PE, Sork VL. 2004. Measuring pollen flow in forest trees: an exposition of alternative approaches. *Forest Ecol Manag*. 197:21–38.

Thiele J, Hansen T, Siegismund HR, Hauser TP. 2010. Genetic variation of inbreeding depression among floral and fitness traits in *Silene nutans*. *Heredity (Edinb)*. 104:52–60.

Wandeck FA, Justo PG. 1988. A macaúba, fonte energética e insumo industrial: sua significacão econômica no Brasil. In: *Simposio Sobre O Cerrado, Savana, Brasília*. Embrapa, CPAC, p. 541–577.

Wilcock C, Neiland R. 2002. Pollination failure in plants: why it happens and when it matters. *Trends Plant Sci*. 7:270–277.

Winn AA, Elle E, Kalisz S, Cheptou PO, Eckert CG, Goodwillie C, Johnston MO, Moeller DA, Ree RH, Sargent RD, *et al.* 2011. Analysis of inbreeding depression in mixed-mating plants provides evidence for selective interference and stable mixed mating. *Evolution*. 65:3339–3359.

Zapata TR, Arroyo MTK. 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. *Biotropica*. 10:221–223.

Zona S, Henderson A. 1989. A review of mediated seed dispersal of palms. *Selbyana*. 11:6–21.