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Mating system analysis of Açaí-do-Amazonas (*Euterpe precatoria* Mart.) using molecular markers

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Abstract: *Euterpe precatoria (Açaí-do-Amazonas) produces fruits of which the fresh pulp is consumed. It is almost exclusively collected by extractivist farmers, because no selected genotypes are available for the establishment of plantations. For the domestication and breeding of the species, mating system studies are needed for strategy formulation. This study evaluated the mating system of a natural population of E. precatoria. Thirteen progenies were genotyped with 13 microsatellite loci by capillary electrophoresis in an automated DNA sequencer. Estimates of single-locus and multilocus outcrossing rates were 1.0, and paternity correlation was low ($\hat{r}_{p(m)} = 0.293$). Euterpe precatoria families consist mainly of half-sibs and the reproductive strategy of the species is allogamy.*

Keywords: *Arecaceae, Euterpe precatoria, molecular marker.*

INTRODUCTION

The palm *Euterpe precatoria* Mart. (acaí-do-Amazonas) belongs to the family Arecaceae, occurring in the western and central Brazilian Amazon and to within borders of the Amazon of Peru, Brazil, Colombia (Kahn 1991), and Bolivia (Bussmann and Zambrana 2012). The species is exploited by an extractive production chain, maintained by the local population since several decades and currently intensified due to the interest of different industries. Fruit for consumption in the form of beverage is the main product of *E. precatoria* (Noda 2012).

When correlating the total production of açaí fruits exploited in the different states of Brazil (IBGE 2018) with the information of geographic distribution and habitat (Lorenzi et al. 2010), it becomes clear that açaí from the states of Amazonas, Acre and Rondônia is extracted from fruits of *E. precatoria*. However, in the states of Pará, Maranhão and Amapá, it is extracted from *Euterpe oleracea,* another açaí species. *Euterpe precatoria* is single-stemmed, while *E. oleracea* has tillers (multi-stemmed). One of the advantages of *E. precatoria* is that the fruit pulp has better anti-oxidant and anti-inflammatory properties than *E. oleracea* (Kang et al. 2012). Moreover, the evolutionary adaptation to nutrient-poor and well-drained lowland, Latosolos and Argisolos is noteworthy

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(FAO 1987). The adaptation of *E. precatoria* to the Amazonian ecosystem indicates it for exploitation in the recovery of degraded areas, although no seeds of improved varieties recommended for the production of seedlings and planting are available, since to date, *E. precatoria* has been neglected in research.

The inflorescences of *E. precatoria* have numerous male (4.5 x 2.7 mm) and female (3.2 x 2.6 mm) flowers. The male flowers open and release pollen before the female flowers are receptive (Küchmeister et al. 1997, Lorenzi et al. 2010). However, this observation of protandry is no guarantee that the species is autogamous, since nonetheless some crosses may occur. Thus, research must be carried out to fill gaps in the knowledge about the gene flow and mating system to interpret its behavior within tropical forest ecosystems in *in situ* or *ex situ* conservation areas.

Euterpe precatoria is a highly important species for the Amazon region. As the genetic structure of tree populations is partially determined by the mating system and strongly by the gene flow among populations (Ramos et al. 2016a), this study will provide valuable information for the domestication and rational management of the species (Ramos et al. 2011). Studies of the mating system can be based on microsatellite markers or simple sequence repeats (SSRs), which are an adequate tool for this purpose, due to the high polymorphism in terms of number of alleles, co-dominant inheritance and low cost of the method (Wadt et al. 2015). Simple sequence repeat-based studies to determine the mating system were carried out for different Amazonian and tropical tree species (Ramos et al. 2011, Medina-Macedo et al. 2015, Picanço-Rodrigues et al. 2015; Moraes et al. 2018).

The objective of this study was to investigate the mating system of a natural *E. precatoria* population, to identify the levels of outcrossing, mating among relatives and correlated matings to better understand the genetic structure of open-pollinated progenies for *ex situ* conservation and breeding plans. Moreover, we tried to determine the coancestry coefficient, effective population size within progeny and the estimated number of trees to ensure enough seeds for conservation.

MATERIAL AND METHODS

Study area and sampling

The study investigated a natural *E. precatoria* population in the community of Nossa Senhora de Fatima do Açaí, in the rural area of Vila Amazônia (lat 02º 36' 52.09" S, long 56º 33' 29.13" W, and alt around 40 m), in Parintins, state of Amazonas, Brazil, where the climate is tropical monsoon (Am) (Peel et al. 2007). All 13 trees bearing fruit within 10 hectares of climax forest were used for the study. Plant material (leaflets) and 100 fruits per mother tree were collected, i.e., a total sample of 1300 fruits. The fruits were transported to the seed laboratory of the experimental field Caldeirão of Embrapa Western Amazon, located at Rodovia Manoel Urbano, Km 13, Estrada do Caldeirão, Iranduba-AM, for seed germination, emergence and seedling production. To accelerate germination, fruits with pulp and seeds were immersed in water at 60 °C and removed when the water reached room temperature (Nogueira et al. 1995). After three months, seedlings of all progenies with at least two bifid leaves were sampled for DNA extraction and microsatellite analysis, resulting in 3 to 25 seedlings per progeny, totaling 227 seedlings (Table 2). One leaflet of each seedling (227 samples) and mother tree (13 samples) was collected and packed separately in a previously labelled zip lock plastic bag containing silica gel. These samples were transported to the Laboratory of Genetics and Plant Breeding of the Department of Agronomy of the Federal University of Amazonas (UFAM) for storage at -20 °C for further genomic DNA isolation.

Amplification of microsatellite markers

The DNA was extracted based on the Cationic Hexadecyl Trimethyl Ammonium Bromide (CTAB) method described by Doyle and Doyle (1990) and then quantified (Ramos et al. 2011). A total of 13 microsatellite primers (Epr01, Epr02, Epr04, Epr05, Epr13, Epr14, Epr15, Epr15, Epr18, Epr19, Epr21, Epr22, Epr31, and Epr32) were used in the study (Ramos et al. 2016b). These microsatellite loci were amplified by the polymerase chain reaction (PCR) with a Veriti thermal cycler (Applied Biosystems) in a total volume of 10 µL per reaction (containing 10 ng of genomic DNA, 1X buffer, 210 µM of each dNTP, 1.5mM $MgCl_2$, 0.16 µM forward primer and M13 label primers (FAM or NED) broth (Schuelke 2000), 0.32 µM reverse primer, 1.05 U Taq DNA polymerase (Invitrogen, Carlsbad, California, USA), and 3.49 µl ultra-pure water. These PCR amplifications consisted of two steps, of which the first was primer-specific and the second with M13 binding (Ramos et al. 2016b). The amplification products were checked by electrophoresis on 1.5% agarose gels stained with

GelRed (Biotium) in 1x TBE buffer (pH 8.0). The amplified PCR products were subjected to an automated DNA analyzer by capillary electrophoresis (ABI 3130XL, Genetic Analyzer, Applied Biosystems). A standard size GeneScan ™ -500 ROX® (Life Technologies of Brazil Ltda.) was used to determine the allele size. The amplified fragments were observed and analyzed with software GeneMapper v4.0 (Applied Biosystems).

Data analysis

The mating system was analyzed using mixed mating and correlated mating models with software MLTR 3.4 (Ritland 2004). Analyses were based on probabilities of maximum expectations "ME". The following indices were estimated: multilocus outcrossing rate ($\hat{t}_m$), single-locus outcrossing rate ($\hat{t}_s$), biparental inbreeding or mating between relatives ($\hat{t}_m - \hat{t}_s$), multilocus paternity correlation ($\hat{r}_{p(m)}$), selfing correlation ($\hat{r}_s$) and maternal fixation index ($\hat{F}_m$). The t_m of each family was estimated by the Moment method. The 95% confidence interval (CI) of each index was calculated from 1000 bootstrap replications, where the sampling units were represented by plants within progenies for the individual analysis and by progenies for population analysis. The mean effective number of pollen donors was estimated by $\hat{N}_{e(p)} = 1/r_{p(m)}$ (Ritland 1989) and the average proportions of pairwise self-sibs ($\hat{P}_{ss}$), half-sibs ($\hat{P}_{hs}$), full-sibs ($\hat{P}_{fs}$), and self-half-sibs ($\hat{P}_{shs}$) within families were estimated as: $\hat{P}_{ss} = \hat{s}^2$; $\hat{P}_{hs} = \hat{t}_m^2(1-\hat{r}_{p(m)})$; $\hat{P}_{fs} = \hat{t}_m^2\hat{r}_{p(m)}$ and $\hat{P}_{shs} = 2\hat{s}\hat{t}_m$ (Sebbenn 2006, Wadt et al. 2015). The coancestry coefficient within family (Θ) was estimated by $\Theta = 0.125(1+\hat{F}_m)[4\hat{s} + (\hat{t}_m^2 + \hat{t}_m\hat{s}\hat{r}_s)(1+\hat{r}_{s(m)})]$, where *s* is the selfing rate ($\hat{s} = 1-\hat{t}_m$) and variance effective size within family, assuming an idealized reference population (infinite size, random mating, without selection, mutation or migration): $\hat{N}_e = 0.5/\{\Theta[(n-1/n)]+[(1+\hat{F}_o)/2n]\}$, where *n* is the number of analyzed offspring within families (we used the average of 227 seedlings, n= 17.46) and $\hat{F}_o$ is the level of inbreeding within families (Sebbenn 2006). The number of mother trees (*m*) for seed collection, aiming to retain the reference effective population size of 150 was calculated by $m = N_{e(reference)}/N_e = 150/N_e$ $m = \hat{N}_{e(reference)}/\hat{N}_e = 150/\hat{N}_e$ (Sebbenn 2006), based on three assumptions: i) non-relatedness of the mother trees; ii) no intermating of the sampled mother trees; iii) no overlapping of the pollen pools and no pollen from same fathers received by the mother trees (Sebbenn 2006).

RESULTS AND DISCUSSION

The maternal fixation index ($\hat{F}_m$) was not different from zero, indicating no inbreeding level of the mother trees. A similar result was found for *Malpighia emarginata* (Lopes et al. 2002). The multilocus outcrossing rate ($\hat{t}_m$) and single-locus outcrossing rate ($\hat{t}_s$) were equal to 1 (Table 1), indicating that the progenies were originated by outcrossing and that the species is allogamous. A similar finding was reported for the palm *Astrocaryum aculeatum* (Ramos et al. 2011). The difference between the multilocus and the single-locus outcrossing rate measured as the mating rate among related individuals ($\hat{t}_m - \hat{t}_s$), was zero (0.001), evidencing the absence of mating between relatives of the sampled seedlings.

The estimated selfing correlation ($\hat{r}_s$) among progenies was low (0.11), indicating low variation in $\hat{t}_m$ among mother trees. Accordingly, the $\hat{t}_m$ among seed trees ranged from 0.79 to 1, in other words, it was significantly lower than 1.0 in 11 of the 13 families (Table 2), showing that some seedlings were originated by inbreeding. This result also suggests that the species is self-incompatible (Moraes et al. 2018). The individual variation in the outcrossing rate may be attributed to flowering asynchrony.

The paternity correlation ($\hat{r}_{p(m)}$) was moderate (0.293) and indicated that about 3.4 effective pollen donors ($\hat{N}_{e(p)}$) fertilized the seed trees in the investigated reproductive event (Table 1). These results show that the families are mainly composed of half-sibs (71%) and that mating was not random. The highest $\hat{N}_{e(p)}$ was reported for the palm *A. aculeatum* (5.7, Ramos et al. 2011). Correlated matings can be caused by the behavior of pollinators that systematically visit nearby trees (Spoladore et al. 2017). Non-random matings were also evidenced by the variation

Table 1. Mating system at the population level

Parameters	**Mean** (95% CI)
Maternal fixation index: $\hat{F}_m$	0 (0–0)
Multilocus outcrossing rate: $\hat{t}_m$	1.0 (1.0–1.0)
Single-locus outcrossing rate: $\hat{t}_s$	0.999 (0.999–1.0)
Mating among relatives: $\hat{t}_m - \hat{t}_s$	0.001 (0.001–0.000)
Selfing correlation: $\hat{r}_s$	0.11 (0.11–0.11)
Paternity correlation: $\hat{r}_{p(m)}$	0.293 (0.127–0.347)
Number of pollen donors: $\hat{N}_{e(p)}$	3.4(2.9–7.9.)
Frequency of self-sibs: $\hat{P}_{ss}$	0 (0–0)
Frequency of self-half-sibs: $\hat{P}_{shs}$	0 (0–0)
Frequency of half-sibs: $\hat{P}_{hs}$	0.71 (0.65–0.87)
Frequency of full-sibs: $\hat{P}_{fs}$	0.29 (0.13–0.35)
Coancestry within family:Θ	0.162 (0.141–0.168)
Variance effective size: $\hat{N}_e$	2.76 (2.67–3.10)
Number of seed trees: $\hat{m}$	54 (48–55)

95% CI - confidence interval 95%.

Table 2. Mating system at the mother tree level

Mother	*n*	$\hat{t}_m$ (SD)	$\hat{t}_m - \hat{t}_s$ (SD)	$\hat{r}_{p(m)}$ (SD)	$\hat{N}_{e(p)}$	$\hat{\Theta}$	$\hat{N}_e$
Family 1	12	0.95 (0.03)*	0.31 (0.04)*	0.44 (0.08)*	2.3	0.251	1.86
Family 2	11	1.00 (0.00)	0.11 (0.02)*	0.08 (0.01)*	11.9	0.136	3.49
Family 3	3	0.97 (0.02)*	0.08 (0.02)*	0.09 (0.01)*	11.8	0.142	3.25
Family 4	22	0.98 (0.01)*	0.08 (0.02)*	0.18 (0.05)*	5.7	0.150	3.03
Family 5	25	0.95 (0.01)*	0.05 (0.01)*	0.11 (0.01)*	9.4	0.150	2.50
Family 6	22	0.79 (0.07)*	0.08 (0.05)*	0.15 (0.05)*	6.7	0.195	2.42
Family 7	9	0.99 (0.01)	0.10 (0.01)*	0.10 (0.00)*	9.8	0.141	3.22
Family 8	22	0.94 (0.04)*	0.15 (0.04)*	0.11 (0.03)*	8.8	0.171	2.61
Family 9	10	0.94 (0.04)*	0.07 (0.03)*	0.15 (0.04)*	6.8	0.157	2.80
Family 10	22	0.99 (0.01)	0.11 (0.02)*	0.12 (0.04)*	8.4	0.155	3.03
Family 11	25	0.88 (0.05)*	0.35 (0.05)*	0.58 (0.08)*	1.7	0.295	1.63
Family 12	22	0.98 (0.01)*	0.13 (0.02)*	0.19 (0.06)*	5.3	0.184	2.51
Family 13	22	0.96 (0.03)*	0.15 (0.03)*	0.13 (0.04)*	7.7	0.150	3.06

n: sample size; $\hat{t}_m$: multilocus outcrossing rate; $\hat{t}_m - \hat{t}_s$: mating among relatives; $\hat{r}_{p(m)}$: paternity correlation; $\hat{N}_{e(p)}$: effective number of pollen donors; $\hat{\Theta}$: coancestry coefficient; $\hat{N}_e$: effective size; SD: standard deviation, calculated from 1000 bootstrap samples. *P < 0.05

among families for mating among relatives ($\hat{t}_m - \hat{t}_s$: between 0.05 - 0.35), paternity correlation rates ($\hat{r}_{p(m)}$: 0.08 - 0.58) and effective number of pollen-donor trees ($\hat{N}_{e\,(p)}$: 1.7 - 11.9, Table 2).

The mean coancestry coefficient within progenies (Θ = 0.162) was higher than expected in half-sib progenies (0.125). Thus, estimates of addictive genetic variance and heritability must be calculated using a relatedness coefficient (Sobierajski et al. 2006) of 0.324 (2Θ) instead of 0.25. Knowledge about the coancestry coefficient is also important when estimating the variance effective size ($\hat{N}_e$), which was lower ($\hat{N}_e$ = 2.76) than expected in the random mating populations (4, Furlani et al. 2005). In infinite samples of progeny structures, the variance effective size varies from 1 to 4, where value 1 indicates selfed progenies, 2 full-sibs and 4 half-sib progenies (Sebbenn 2006). Due to the estimated effective population size, the seeds must be collected from at least 54 trees to retain the effective reference population size of 150 in progeny array samples. These analyses are important for estimating sample sizes in breeding, genetic conservation and seed collection programs addressing environmental recovery, as well as for the monitoring of genetic diversity in manipulated populations (Moraes et al. 2018).

CONCLUSION

The mating system indices estimated for *E. precatoria* in this study indicated that the species is allogamous but self-compatible. The studied progenies were mainly represented by half-sibs, but matings were not random due to the occurrence of some correlated mating, resulting in a few full-sibs within progenies.

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