ISSN 0104-7760

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CONTRASTING LEVELS OF GENETIC DIVERSITY AMONG POPULATIONS OF THE ENDANGERED TROPICAL PALM *EUTERPE EDULIS* MARTIUS

Keywords:
Genetic structure
Inbreeding
Outcrossing rate
Genetic distance
Isozymes

ABSTRACT: *Euterpe edulis* is a tropical species that produces the heart of palm, an edible product of high economic importance. However, its natural populations have been severely threatened by unrestricted exploitation, along with the destruction of its natural biome, the Atlantic Rainforest in Brazil. In this work, we examined the genetic diversity status of five natural populations using isozyme markers. Despite their limitations and replacement by DNA-based markers, isozymes are codominant markers that reveal accurate estimates of genetic diversity and structure patterns, as do microsatellites. Six informative isozyme markers were used to analyze the genetic variability of populations located in different areas of the Atlantic Forest (Ombrophilous Dense Forest and Seasonal Forest), and with different degrees of perturbation. Mean genetic diversity for all populations (H_o = 0.172, for 13 loci) was considered low for a tropical species, even for the markers used. Populations from Ombrophilous Dense Forest at the very South limit of distribution of the heart of palm presented the lowest genetic variability (H_o = 0.141), which is clearly observed from the allele frequencies, and might implicate in less adaptive potential in a scenario of climate change. On the contrary, the Seasonal Forest population presented the highest diversity (H_o = 0.237). It comprises one of the largest remaining reservoirs of heart of palm and maybe of its genetic variability. The contrasting levels of genetic diversity encountered in this study rehash the constant need of monitoring and conserving the current genetic diversity of *E. edulis* populations, as well as exploring strategies for its breeding.

Histórico:
Recebido 25/09/2016
Aceito 24/12/2016

NÍVEIS DE DIVERSIDADE GENÉTICA CONTRASTANTES ENTRE POPULAÇÕES DE EUTERPE EDULIS MARTIUS, PALMEIRA TROPICAL AMEAÇADA

Palavras chave:
Estrutura genética
Endogamia
Taxa de intercruzamento
Distância genética
Isoenzimas

RESUMO: Euterpe edulis é uma espécie tropical que produz o palmito, produto comestível de elevada importância econômica. No entanto, as populações naturais da espécie têm sido severamente ameaçadas devido à exploração indiscriminada, conjuntamente com a destruição de seu bioma natural, a Mata Atlântica. Apesar de suas limitações e substituição por marcadores de DNA, isoenzimas são marcadores codominantes que permitem obter estimativas acuradas dos padrões de diversidade e estrutura genética, assim como microssatélites. Seis marcadores isoenzimáticos foram utilizados para analisar a variabilidade de populações localizadas em diferentes áreas da Mata Atlântica (Floresta Ombrófila Densa e Floresta Estacional), e com diferentes níveis de perturbação. A diversidade genética média de todas as populações (*Ho* = 0,172, para 13 locos) foi considerada reduzida para uma espécie tropical, até mesmo para os marcadores empregados. Populações da Floresta Ombrófila Densa no limite sul de distribuição do palmiteiro apresentaram a diversidade genética mais reduzida (*Ho* = 0,141), o que é claramente observado a partir de perfis de peroxidase, e pode implicar em menor potencial adaptativo em um cenário de mudança climática. Contrariamente, a população da Floresta Estacional apresentou a diversidade mais elevada (*Ho* = 0.237). Esta população compreende um das maiores reservas de palmiteiro e, talvez, de sua diversidade genética. Os valores de diversidade encontrados neste estudo reforçam a necessidade contínua do monitoramento e conservação da diversidade genética atual das populações de E. edulis, assim como o desenvolvimento de estratégias para seu melhoramento.

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DOI:
10.1590/01047760201723012237

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INTRODUCTION

Euterpe edulis Martius (2n = 2x = 36 chromosomes) (OLIVEIRA et al., 2016), known as heart of palm or palm heart, is native to the Atlantic Rainforest (REIS et al., 2000a; CONTE et al., 2008). Originally, *E. edulis* was found in high density and abundance in the middle stratum of the Atlantic Forest. The species spread in a disjointed manner in two distinct areas. One is the Ombrophilous Dense Forest, from Northeastern to Southern Brazil and part of the Seasonal Forests from the South. The other area comprises the Seasonal Forests in Paraná and Uruguay River Basins, spreading through Central-Western to Southern Brazil and extending to Eastern Paraguay and Northern Argentina (REIS et al., 2000a).

Currently, the remaining populations are restricted to the coastal rainforests in protected or private areas. In the Seasonal Forest, one of the largest reservoirs of heart of palm is the National Park of Iguaçu (Foz do Iguaçu, Paraná), which is the largest continuous area still with high density of the species (REIS et al. 2000a). In Southern Brazil, remnant populations occur mainly in small forest fragments (MARTINS-CORDER et al., 2009), as well as in the Southeastern region of the country (OLIVEIRA et al., 2015; CARVALHO et al., 2015). Most of the remnant populations possibly had significant genetic erosion, which might be due to random genetic drift and the fragmented landscape (REIS et al., 2000b; VIEIRA et al., 2010; OLIVEIRA et al., 2015; CARVALHO et al., 2015).

The fragmentation of *E. edulis* populations has been aggravated by the intense exploitation that has been destroying the Atlantic Forest (CARVALHO et al., 2015), which is currently reduced to less than 12% of its original coverage (RIBEIRO et al., 2009). Moreover, the high perturbation degree of *E. edulis* populations is mainly due to the intensive extraction of its edible products. First, it produces the high quality palm heart that has been processed into preserves for marketing industries for more than 50 years. The extraction of the palm heart requires the whole plant to be cut. Exhibiting no tillering ability, the individual no longer survives (REIS; REIS, 2000). Second, increased attention has been given to its medicinal potential, such as the antioxidant properties of its fruit (CARDOSO et al., 2015).

The economic importance of the species has increased its demand on the national and international market. Massive exploitation has been occurring, reducing the size of populations or in extreme cases even extinguishing them in small sites (CONTE et al., 2006). Consequently, legislation and government programs aimed at developing strategies for sustainable management of the species have been threatened by illegal and indiscriminate exploitation. As a result, *E. edulis* is within the list of critically endangered species (BRASIL, 2008; BRANCALION et al., 2012).

E. edulis essentially reproduces by outcrossing (GAIOTTO et al., 2003). It has panicle inflorescences with flowers that exhibit strong protandry. Flowers are unisexual and distributed in triads, with one female flower between two male. Pollination is favored by abundant insect fauna, attracted by the high production of pollen and nectar (MORELLATO; MANTOVANI, 2000). Heart of palm has an equally important role in feeding other animals, especially small rodents (REIS; KAGEYAMA, 2000). Therefore, it is considered as a keystone species in the ecological dynamics of the Atlantic Forest (REIS; KAGEYAMA, 2000).

The ecological and economic importance, along with the imminent risk of extinction of *E. edulis*, have required studies on the status of the genetic diversity of its natural populations, in order to design proper conservation strategies. A number of different population genetic studies have accessed the genetic variation and structure of *E. edulis* based on several molecular markers, such as isozymes (REIS et al., 2000b; CONTE et al., 2003; CONTE et al., 2008; MARTINS-CORDER et al., 2009; VIEIRA et al., 2010), AFLP (CARDOSO et al., 2000) and microsatellite markers (GAIOTTO et al., 2003; CONTE et al., 2006; CONTE et al., 2008; OLIVEIRA et al., 2015). Most of the studies, however, were performed in the last decade. Only a few have been published in recent years (VIEIRA et al., 2010; CARVALHO et al., 2015). Some studies have reported possible reduction of the genetic diversity of *E. edulis* populations due to habitat loss and fragmentation (MARTINS-CORDER et al., 2009; CARVALHO et al., 2015), but no conclusive evidence has been shown so far.

DNA-based markers have mostly replaced indirect assessment methods of genetic variability such as those based on isozyme profiles. Although having some drawbacks, such as the small number of loci analyzed, the presence of null alleles, and lower levels of polymorphism, isozymes remain suitable markers for analyses that require no extensive genomic sampling (SULKOWSKA, 2012). Isozymes account for precise estimation of the genetic structure of populations as do microsatellites (CONTE et al., 2008; SULKOWSKA, 2012). Population genetic parameters such as outcrossing rates, as well as estimates of genetic

structure (F_{ST}, G_{ST}) have showed similar results by both isozyme and microsatellite analyses (CONTE et al., 2008). Furthermore, evidences on the adaptive role of isozyme polymorphisms in tree species have been shown, altering a previous paradigm that such markers were only selectively neutral (HARTER et al., 2015).

In this work, we assessed the genetic diversity and structure of five populations with different degrees of perturbation, distributed in major forest areas of *E. edulis* distribution, the Seasonal Forest and the Ombrophilous Dense Forest. Two main questions were addressed. (1) Are the levels of genetic diversity different between conserved and fragmented populations? (2) Could human interference have already altered the genetic diversity and structure of the populations?

MATERIAL AND METHODS

Plant materials and protein extractions

Five populations of *E. edulis* were sampled. The populations are located in areas of Seasonal Forest (National Park of Iguaçu, Foz do Iguaçu, state of Paraná) and Ombrophilous Dense Forest (Maquiné and Dom Pedro de Alcântara, in the coastal region of the Southern state of Rio Grande do Sul; and Tinguá and Petropolis, in the mountains of the state of Rio de Janeiro) (Figure 1). Seeds were collected from at least 10 adult trees of *E. edulis* in each population and planted in black plastic bags containing a mixture of soil and organic substrate (1:1, v/v). The plants were grown in greenhouse conditions. In case of all populations, 160 seedlings were sampled per population for the isozyme analyses. In total, 800 individuals were analyzed.

Leaf tissues were collected from each seedling and ground with 1 mL of extraction solution adapted from Alfenas et al. (2006). The solution was prepared with disodium phosphate (0.034 M), sucrose (0.2 M), polyvinylpyrrolidone (PVP-40, 2.6%, $w v^{-1}$), dithiothreitol (3 mM), L-ascorbic acid (6 mM), diethyldithiocarbamic acid (5.8 mM), sodium bisulfite (2.6 mM), sodium borate (2.5 mM), 2-mercaptoethanol (0.2%, $w\ v^{-1}$), polyethylene glycol 8000 (1%, $w\ v^{-1}$) (ALFENAS et al., 2006). A small trace of polyvinylpolypyrrolidone (PVPP) was added to prevent oxidation and increase protein stability (MARTINS-CORDER; LOPES, 1997). The extracts were absorbed on Whatmann 3 filter paper wicks (5 x 12 mm), placed in Eppendorf microtubes and stored at -18°C for at maximum 15 days.

FIGURE 1 Location of five populations of Euterpe edulis Martius analyzed through six isozyme systems. Dom Pedro de Alcântara, Maquiné, Tinguá and Petrópolis are located in areas of Ombrophilous Dense Forest, while the population of Foz do Iguaçu is located in a Seasonal Forest area.

Electrophoresis and isozyme systems

The electrophoresis was performed with cornstarch gel (Penetrose 30, viscosity 53973') at 13%, using two different buffer systems: Tris-Citrate (TC) and Citrate-Morpholine (CM) (Table 1). The systems were selected due their better performance for each isozyme analyzed in this work. The electrophoresis was conducted in cold chamber at 5°C, with the following conditions: (i) 150 V and 20 mA during approximately 8 hours for the TC system; and (ii) 250 V and 25 mA for approximately 10 hours for the CM system. After electrophoresis, the gels were cut into slices and each slice was placed in a different porcelain tray containing the appropriate solution for revealing each enzyme system. For each individual, six isozyme systems were analyzed (Table 1).

The genetic interpretation of the electrophoretic profiles was performed according to the quaternary structure of each enzyme and their apparent segregation. For each enzyme system, the most anodal isozyme was designated as the first locus, and the others were numbered consecutively. In each locus, the band with more mobility was designated as A_1 allele. The other alleles were numbered consecutively. Diploid genotypes were recorded (homozygotes and heterozygotes) and converted into allele frequencies.

Population diversity and structure analyses

To verify and compare the levels of genetic diversity, POPGENE version 1.31 was used to compute

TABLE 1 Isozyme nomenclature and gel buffer systems used.

Isozyme system	EC number	Buffer system
α-Esterase (α-EST)	E.C.3.1.1.1	TC*
6-Phosphogluconate dehydrogenase (6-PGDH)	E.C.1.11.4.4	CM*
Phosphoglucomutase (PGM)	E.C.5.4.2.2	CM
Phosphogluco isomerase (PGI)	E.C.5.3.1.9	CM
Malate dehydrogenase (MDH)	E.C.1.1.1.37	CM
Peroxidase (PO)	E.C.1.11.1.7	TC

*The buffer systems used were Citrate-Morpholine (CM) – cube: citric acid 0.04 M, adjusted to pH 6.1 com N-3-aminopropil-morpholine; gel: 1:20 dilution factor from the cube buffer (CLAYTON; TRETIAK, 1972); Tris-Citrate (TC) – cube: tris 0.223 M, citric acid 0.086 M, adjusted to pH 7.5; gel: dilution to 3.5% of the cube buffer (JARRET; LITZ, 1986).

the genetic diversity and structure estimates for each population, as well as the joint analysis of all populations (YEH; BOYLE, 1999). The analyses were based on two approaches: (i) all loci; (ii) only the polymorphic loci (CONTE et al., 2003). The following parameters were determined: average number of alleles per locus (A = total number of alleles per polymorphic locus / number of polymorphic loci) and the percentage of polymorphic loci (P = number of polymorphic loci / total number of loci). P was estimated with the criterion of 95% of probability, which considers as polymorphic the locus whose most frequent allele does not exceed 95%. The genetic diversity analysis was also computed based on the average observed heterozygosity ($H_o = \sum(\sum h_{ij}/n)]/l$), where h_{ij} corresponds to the frequency of heterozygotes in the population, n is the number of individuals and l is the number of loci. The other estimate was the average expected heterozygosity under Hardy-Weinberg Equilibrium ($H_e = 2n\ (1 - \sum p_i^2)/\ (2n - 1)$), where p_i is the frequency of the i^{th} allele. The fixation index was estimated according to Wright (1978): $F = 1 - (H_o / H_e)$. From the fixation index was derived the apparent outcrossing rate: $t_a = (1 - F)/(1 + F)$ (WRIGHT, 1921).

To verify a possible reduction in the population effective size of each population (bottleneck), we used the software Bottleneck (PIRY et al., 1999). The analysis was performed using the infinite allele model (IAM) (KIMURA; KROW, 1964). The IAM considers each mutation as generating a new allele, assuming random mating and mutation and drift equilibrium before a bottleneck (CORNUET; LUIKART, 1996). The Wilkoxon signed rank test with 1,000 permutations was performed to verify the significance of the adjustment to the IAM.

The genetic structure was determined under two approaches. First, the F statistics of Wright (1965) which refer to the random analysis model, in which $F_{IT} = F_{ST}\ (1 - F_{ST})\ F_{IS}$. Second, the fixed model analysis model of Nei (1973) was used to estimate the total genetic diversity (H_T), and the components of diversity within populations (H_S) and the proportion of genetic diversity among populations (G_{ST}). From F_{ST} was derived the apparent gene flow ($Nm = (1/4)\ ((1/F_{ST})-1)$).

Non-biased genetic distances of Nei (1978) were estimated among the five populations. The genetic distances were used to generate a dendrogram, using the clustering method UPGMA (Unweighted Pair Group Method with Arithmetic Averages).

RESULTS

Allele frequencies

In total, 15 loci were detected from the six isozyme systems. However, only 13 were considered for the analysis, as *Po-3* presented high percentage of null alleles, which might implicate in errors on the estimates of genetic diversity. *Po-4* was discarded from the analysis, as no appropriate resolution was detected. Considering the average of all populations, eight loci were polymorphic according to the 95% probability criterion (Table 2).

Considering the 13 loci, in total, 42 alleles were detected (Table 2). Rare alleles were observed in the five populations. The allele A_3 of *Po-1* presented low frequency in all populations ($P < 0.05$). The allele A_3 from *Est-1* appeared in low frequency in Maquiné (0.9%), Foz do Iguaçu (2.8%) and Petrópolis (0.9%). A reduced frequency of the allele A_4 of *Pgm* was detected in Dom Pedro de Alcântara (0.3%), Foz do Iguaçu (0.6%) and Petrópolis (0.6%). A_3 of *6-Pgdhh-1* was detected in low frequency in the same populations. At the locus *6-Pgdh-2*, the alleles A_4 and A_5 were detected with frequency lower than 5%.

Private alleles were observed in the populations of this study as well. The alleles A_2 and A_3 of the loci *Po-3* and *Est-5* were private to the populations located in Rio de Janeiro. The allele A_2 from *Mdh-2* was private to Tinguá. The alleles A_3 of *Est-3* and A_3 of *Pgi-2* were only encountered in Foz do Iguaçu. The allele A_6, of *6-Pgdh-2* was private to Maquiné (Table 2).

Genetic diversity levels

The genetic diversity levels were estimated under two approaches: the 13 loci examined in this work; and only the eight polymorphic loci (CONTE et al., 2003) (Table 3). Contrasting levels of diversity were found in each population under both approaches, although higher values were detected when only polymorphic loci were examined.

TABLE 2 Allele frequencies of five populations of Euterpe edulis Martius, basead on 13 isozyme loci.

Loci	Alleles	Populations				
		Maquiné	Dom Pedro	Foz do Iguaçu	Petrópolis	Tinguá
N*	-	160	160	160	160	160
Po-1	1	0.8938	0.9906	0.9094	0.8270	0.6937
	2	0.0594	0.0031	0.0281	0.1069	0.2437
	3	0.0250	0.0063	0.0125	0.0126	0.0563
	4	0.0219	-	0.0500	0.0535	0.0063
Po-2	1	0.9906	0.9938	0.9500	0.9308	0.9219
	2	-	0.0063	0.0500	0.0566	0.0656
	3	0.0094	-	-	0.0126	0.0125
Po-5	1	1.0000	1.0000	1.0000	0.8459	0.9500
	2	-	-	-	0.1509	0.0344
	3	-	-	-	0.0031	0.0156
Est-1	1	0.8844	0.9531	0.7188	0.8868	0.9187
	2	0.1062	0.0469	0.2531	0.1038	0.0813
	3	0.0094	-	0.0281	0.0094	-
Est-2	1	0.9156	0.9938	0.8500	0.9686	0.9938
	2	0.0844	0.0063	0.1313	0.0314	0.0063
	3	-	-	0.0187	-	-
Est-3	1	1.0000	1.0000	1.0000	0.9843	0.9750
	2	-	-	-	0.0094	0.0187
	3	-	-	-	0.0063	0.0063
Pgm	1	0.8281	0.7969	0.6312	0.4654	0.6875
	2	0.1500	0.1344	0.2812	0.4654	0.2781
	3	0.0219	0.0656	0.0813	0.0629	0.0344
	4	-	0.0031	0.0063	0.0063	-
Pgi-1	1	0.9406	0.9031	0.9469	0.9654	0.9187
	2	0.0250	0.0938	0.0531	0.0283	0.0688
	3	0.0344	0.0031	-	0.0063	0.0125
Pgi-2	1	0.5844	0.8531	0.2750	0.7484	0.9187
	2	0.4156	0.1469	0.7188	0.2516	0.0813
	3	-	-	0.0063	-	-
6-Pgdh-1	1	1.0000	0.9219	0.7312	0.9811	0.9500
	2	-	0.0750	0.2500	0.0126	0.0500
	3	-	0.0031	0.0187	0.0063	-
6-Pgdh-2	1	0.1344	0.3031	0.1125	0.2547	0.2406
	2	0.4437	0.4594	0.4000	0.3553	0.2594
	3	0.3719	0.1906	0.4625	0.3899	0.4719
	4	0.0125	0.0469	0.0125	-	-
	5	-	-	0.0125	-	0.0281
	6	0.0375	-	-	-	-
Mdh-1	1	0.9344	0.9688	0.9719	0.9308	0.9969
	2	0.0656	0.0312	0.0281	0.0692	0.0031
Mdh-2	1	1.0000	1.0000	1.0000	1.0000	0.9656
	2	-	-	-	-	0.0344

* N = number of individuals sampled per population.

The levels of heterozygosity were low in the Southern population of Dom Pedro de Alcântara (H_o = 0.114 with 13 loci; and H_o = 0.181 with eight polymorphic loci). Conversely, the highest level of diversity was found in Foz do Iguaçu (H_o = 0.237, with 13 loci; and H_o = 0.368 with the eight polymorphic loci). The overall average heterozygosity of the five populations was considered low (H_o = 0.172 with the 13 loci, and H_o = 0.254 with the eight polymorphic loci).

The low genetic variability found in Dom Pedro de Alcântara is observed in Table 2, in which various loci presented alleles almost fixed, i.e., Peroxidase profiles (Figure 2). In comparison to the other populations, much less polymorphism was found within this population of Southern Brazil (Table 2).

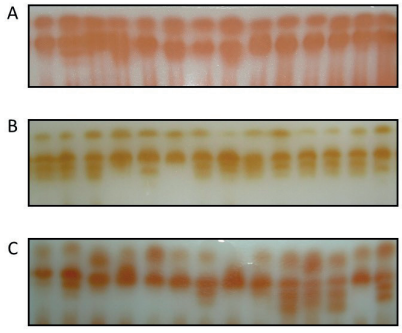

FIGURE 2 Peroxidase profiles of a set of individuals from three populations of Euterpe edulis Martius: (A) Dom Pedro de Alcântara; (B) Foz do Iguaçu; (C) Maquiné.

Overall, moderate levels of inbreeding were found within the populations (F = 0.093, with all loci) (Table 3). The highest levels of inbreeding were detected in Dom Pedro de Alcântara (F = 0.162) and Petrópolis (F = 0.161). Conversely, the fixation indexes were negligible in Maquiné (F = 0.029) and Foz do Iguaçu (F = -0.013). When only polymorphic loci were considered for the analysis, the fixation indexes presented similar values (F = 0.084). Such data along with the goodness of fit test to the Hardy-Weinberg Equilibrium showed most loci are not under equilibrium (Table 4).

The fixation indexes encountered in this study reflected in variable outcrossing rates for the populations analyzed. In average, the apparent outcrossing rate (t_a) was of 83% with all the 12 loci, and of 85% with the eight polymorphic loci. The populations of Foz do Iguaçu (t_a = 103% with all 13 loci and with the eight polymorphic) and Maquiné (t_a = 94% with all loci, and t_a = 99% with only the eight polymorphic loci) presented the highest outcrossing rates.

Bottleneck analysis

The analysis of a possible reduction in the effective population size of the populations was assessed with the Bottleneck software. However, no significant changes (P > 0.05) were verified from the Wilkoxon test for the Infinite Allele Model in each population (P = 0.51, 0.21,

0.42, 0.21, 0.37, respectively for Maquiné, Dom Pedro de Alcântara, Foz do Iguaçu, Petrópolis, Tinguá).

Genetic structure of the populations

The genetic structure analysis revealed that most of genetic diversity was found within populations according to the two approaches used for its determination, the random and the fixed model (F_{ST} = 0.083; G_{ST} = 0.082) (Table 3).

The apparent gene flow (N_m) values were high between Maquiné and Dom Pedro de Alcântara (Rio Grande do Sul state) (N_m = 7.5), and between Petrópolis and Tinguá (Rio de Janeiro state) (N_m = 10.5). Moreover, the gene flow between the populations of Rio Grande do Sul and Rio de Janeiro was higher (N_m = 4.4) than between those groups and Foz do Iguaçu (Seasonal Forest) (N_m varied from 2.8 between Iguaçu and the populations of Rio Grande do Sul, to 3.0 between Iguaçu and the group from Rio de Janeiro). However, the average gene flow among the five populations was lower (N_m = 2.8), reflecting the geographical distance among the populations.

TABLE 3 Mean number of alleles per locus (A), percentage of polymorphic loci (P), average observed heterozigosity (Ho), average expected heterozigosity (He), fixation index (F) and apparent outcrossing rate (ta) of Euterpe edulis Martius populations, considering 13 isozyme loci (A) and only polymorphic loci[1] (B).

Population		*A*	*P%*	*Ho*	*He*	*F*	*ta*
Ombrophilous Dense Forest in Rio Grande do Sul, Southern Brazil							
Dom Pedro de Alcântara	A	2.3	38.4	0.114	0.136	0.162	0.72
	B	2.9	100.0	0.181	0.212	0.146	0.74
Maquiné	A	2.3	61.5	0.167	0.172	0.029	0.94
	B	2.9	100.0	0.262	0.263	0.004	0.99
Mean	A	2.3	50.0	0.141	0.154	0.088	0.84
	B	2.9	100.0	0.221	0.237	0.067	0.87
Ombrophilous Dense Forest in Rio de Janeiro, Southeastern Brazil							
Petrópolis	A	2.8	61.5	0.182	0.217	0.161	0.72
	B	3.0	100.0	0.233	0.284	0.180	0.70
Tinguá	A	2.7	69.2	0.162	0.191	0.152	0.74
	B	2.8	100.0	0.226	0.265	0.148	0.74
Mean	A	2.8	65.4	0.172	0.204	0.157	0.73
	B	2.9	100.0	0.229	0.275	0.164	0.72
Seasonal Forest							
Foz do Iguaçu	A	2.6	69.2	0.237	0.234	-0.013	1.03
	B	3.4	100.0	0.368	0.362	-0.017	1.03
Mean of populations	A	2.5	60.0	0.172	0.190	0.093	0.83
	B	3.0	100.0	0.254	0.277	0.084	0.85

[1]Polymorphic loci (95%): Po-1, Est-1, Est-2, Pgm, Pgi-1, Pgi-2, 6-Pgdh-1 e 6-Pgdh-2.

The genetic distances among the populations of the Ombrophilous Dense Forest (Maquiné, Dom Pedro de Alcântara, Tinguá and Petrópolis) were low, even considering the high geographic distance between Rio de Janeiro and Rio Grande do Sul. On the contrary, the genetic distances between populations from Ombrophilous Dense Forest and from the Seasonal Forest was much higher. From the UPGMA analysis, the populations were coherently grouped according to their geographical distance and the forest type.

TABLE 4 Number of individuals analyzed (*N*), number of degrees of freedom (*DF*), chi-square goodness of fit test for Hardy-Weinberg Equilibrium (χ^2) and probability of equilibrium (*P*), of 13 isozyme loci in *Euterpe edulis* Martius populations.

Locus	N	Hardy-Weinberg Equilibrium goodness of fit test		
		DF	χ^2	*P*
Po-1	160	6	540.946*	<0.01^{ns}
Po-2	160	6	157.511*	<0.01^{ns}
Po-5	160	6	0.248	0.97*
Est-1	160	6	17.598*	<0.01^{ns}
Est-2	160	3	109.535*	<0.01^{ns}
Est-3	160	3	0.049	0.99*
Pgm	160	6	349.770*	<0.01^{ns}
Pgi-1	160	3	988.180*	<0.01^{ns}
Pgi-2	160	6	117.701*	<0.01^{ns}
6-Pgdh-1	160	3	6.938*	0.07^{ns}
6-Pgdh-2	160	15	393.088*	<0.01^{ns}
Mdh-1	160	3	84.611*	<0.01^{ns}
Mdh-2	160	1	27.413*	<0.01^{ns}

*: Significant at 5%. ns: not significant.

TABLE 5 Number of individuals analyzed (*N*), number of degrees of freedom (*DF*), chi-square goodness of fit test for Hardy-Weinberg Equilibrium (χ^2) and probability of equilibrium (*P*), of 13 isozyme loci in *Euterpe edulis* Martius populations.

Populations	F_{IT}	F_{IS}	F_{ST}	Nm**	H_T	HS	GST
Maquiné x Dom Pedro (Group 1)	0.115	0.086	0.032	7.5	0.159	0.154	0.031
Petrópolis x Tinguá (Group 2)	0.172	0.153	0.023	10.8	0.208	0.204	0.019
Group 1 x Foz do Iguaçu	0.117	0.043	0.078	3.0	0.196	0.180	0.082
Group 2 x Foz do Iguaçu	0.166	0.092	0.081	2.8	0.232	0.214	0.078
Group 1 x Group 2	0.171	0.124	0.053	4.4	0.189	0.179	0.053
Joint analysis	0.165	0.090	0.083	2.8	0.207	0.190	0.082

* F_{IS}, F_{ST} and F_{IT} refer to the partition of endogamy within, among and the total of populations, respectively. H_T is the total diversity of populations, Hs is the diversity within populations and G_{ST} is the proportion of the genetic diversity among populations.** Based on F_{ST}.

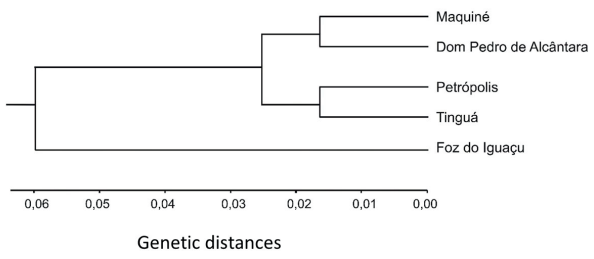

FIGURE 3 Dendrogram based on UPGMA methods (Unweighted Pair-Group with Arithmetic Averages), of *Euterpe edulis* Martius populations, using Nei's non-biased genetic distances, based on 13 isozyme loci.

DISCUSSION

Rare and private alleles within populations

We observed alleles with low frequencies (less than 5%) in the populations. Rare alleles could be an indication of recent mutation events, in this case, on the level of protein phenotypes. As *E. edulis* populations are severely threatened by the constant illegal exploitation along with the continuous reduction of the size of the current forest remnants, such alleles might easily be lost. Although our study has encompassed no statistical tests on the neutrality of the alleles detected, such markers could be of important functional value to current populations adapting to changing conditions (HARTER et al., 2015). The detection of private alleles in this work reinforces the hypothesis, as the populations are located in areas with different climates and forests. New alleles to populations could represent an important adaptation mechanism to climate change, as generations advance. The populations from southern Brazil (Maquiné and Dom Pedro de Alcântara) are subject to frequent rains and a high contrast of temperatures over the year, especially in winter, with cold fronts that occasionally drop temperatures to 0°C or below. Tinguá and Petrópolis (Southeast Brazil, state of Rio de Janeiro) are located in higher altitudes and may as well have high temperature amplitude. These populations contrast with Foz do Iguaçu, which is located in a Seasonal Forest, which has been naturally disconnected from the Atlantic Rain Forest.

Contrasting levels of genetic diversity

The populations analyzed presented contrasting levels of genetic diversity. The lowest heterozygosity was found in the Southern population of Dom Pedro de Alcântara (Ombrophilous Dense Forest) (Table 3). On the contrary, the population of Foz do Iguaçu (Seasonal Forest) presented the highest heterozygosity from the populations in this study.

First, the low diversity observed in populations such as Dom Pedro de Alcântara could be explained by natural events. The population is located at the very southern limit of the natural distribution of *E. edulis* populations. The distribution of the species further south might have been an event related to the post-glacial era, which implied in a progressive warming of Southern areas of Brazil, creating conditions for tropical species to spread further south, as has been reported for *E. edulis* (REIS et al., 2000a). Such event could be related to the establishment of populations with lower effective population sizes, as those determined by demographic and genetic bottlenecks and founder effects. Significance evidence on the matter has been reported for forest tree species (ROBERTS; HAMANN, 2015). However, the Wilkoxon test of the infinite allele model for bottleneck revealed no significant changes in each population size.

Second, the intensive habitat fragmentation, leading to the isolation of populations, along with the dramatic reduction in their size, might have already interfered with the genetic diversity of *E. edulis*. This could be the case in Dom Pedro de Alcântara. The sampling strategy of this work is supportive to this inference, as only seedlings grown from sampled seeds from each population were analyzed. Adults and seedlings may show distinct responses to population fragmentation and reduction in size. While adult individuals often show responses due to past landscape conditions, the analysis of seedling provides data on ongoing processes in these populations (CARVALHO et al., 2015). The study of Carvalho et al. (2015) involved a coalescent analysis of *E. edulis* populations of remnant Atlantic Forest landscapes with distinct conservation status (5 to 75% forest cover), based on the sampling of only seedlings. The analysis indicated high historical migration among populations until recently, suggesting a common evolutionary history. However, recent events of habitat loss and landscape fragmentation might have already reduced the genetic variability, as observed especially in sites with low forest cover (5%). From our study, forest remnants such as Dom Pedro de Alcântara have experienced severe reduction in size and exploitation of heart of palm, probably leading to loss of genetic diversity. Moreover, with a lower number of individuals, the probability of inbreeding and its consequences could be increased, as shown from the fixation indexes of this population (Table 3).

Other studies have discussed the probable effect of human interference on the genetic diversity of natural populations of *E. edulis*. The analysis of other populations from the Southern state of Rio Grande do Sul identified sites with variable values of heterozygosity, some of them with considerably high levels of inbreeding, which could be possibly related to their critical state of fragmentation (MARTINS-CORDER et al., 2009). A microsatellite analysis of 16 sites distributed across five landscapes of the Southeastern state of São Paulo showed a partial association of genetic diversity and inbreeding levels. In general, populations located in areas severely deforested showed lower heterozygosity levels than more conserved populations (CARVALHO et al., 2015).

The population of Foz do Iguaçu, on the contrary, is located in one of the largest conserved areas of Seasonal Forests in the Atlantic Forest. Thereby, it is one of the reservoirs of germplasm of *E. edulis*, which was demonstrated by higher levels of genetic diversity. Other studies have also showed populations with consistently higher values of genetic diversity, even though with some degree of perturbation. A compilation of several population genetic studies with *E. edulis* is presented on Table 6. The studies have been performed across several sites of Brazil and with individuals at different stages (progenies, juveniles and adults).

Genetic structure

Although with variable levels of genetic diversity, the populations maintained low genetic differentiation, as revealed by Wright's and Nei's statistics. Several other studies have shown low genetic differentiation (REIS, 1996; REIS et al., 2000; CONTE, 2004; SILVA, 2004; CONTE et al., 2006; CONTE et al., 2008; MARTINS-CORDER et al., 2009) and considerable gene flow among them (REIS, 1996; REIS et al., 2000; GAIOTTO et al., 2003; MARTINS-CORDER et al., 2009) (Table 6). The levels of gene flow among groups of populations (Table 5) were coherent with the model of isolation by distance (SLATKIN, 1993), as the populations located within small distances presented high levels of gene flow, while geographically distant populations revealed lower values of such parameter. However, the current gene flow may just be a reflex of past generations, with no significant alteration on the balancing of alleles among populations so far, even for the case of seedlings sampled in this study. Habitat loss and fragmentation of *E. edulis* throughout most of its spanning area have the potential to increase the genetic differentiation and reduce the gene flow over the next generations (CARVALHO et al., 2015).

Implications of the results

Our results have important implications for the conservation of *E. edulis*. With the reduced genetic divergence detected among the populations, sampling of genetic materials might be performed only in a few populations, as has been demonstrated by other studies as well (Table 6).

However, one major concern from our results is related to *in situ* conservation approaches. The low genetic diversity presented by the Southern population of Dom Pedro de Alcântara suggests the introduction of alternative genotypes and alleles from other locations for raising its levels of genetic diversity. Such action may reduce inbreeding levels and give conditions for the survival of the population in a scenario of climate change.

The high genetic diversity of Foz do Iguaçu is a potential source for selecting genetic materials for breeding programs of the species. Further studies on the genetic potential of genotypes from this population could allow the selection of individuals with superior performances for heart of palm and fruit production. The seeds of such individuals might be used for breeding purposes and should be adequately stored in germplasm banks.

CONCLUSIONS

Contrasting levels of genetic diversity were encountered among five populations of *E. edulis* distributed throughout remnant areas of the Atlantic Forest. The Southern population of Dom Pedro de Alcântara revealed low genetic diversity, which could be related to natural events, however, no significant reduction in the population effective size was detected considering the infinite allele model. The current fragmented landscape and reduced size of the population, however, suggest human interference might have already interfered with the diversity levels at this site. On the other hand, the population of Foz do Iguaçu revealed high genetic diversity. The area encompasses a large reservoir of *E. edulis* genetic resources that should be thoroughly conserved over the next generations.

Despite the contrasting diversity levels, low genetic divergence was detected among the populations. Conservation *ex situ* might be performed with the selection of genotypes of only a few populations. Conversely, *in situ* conservation approaches are required, especially for populations with low genetic diversity. Alternative genetic materials should be introduced to populations with low genetic diversity.

The continuous monitoring of the genetic diversity and structure of populations of *E. edulis* is necessary in a scenario of climate change and non-stopping human interference to the environment. Modern approaches of sequencing

TABLE 6 Compilation of population genetic studies of Euterpe edulis populations, based on molecular markers.

Genetic marker	Conservation status of the populations and sampling	Number of populations	*A*	*P*	*Ho*	*He*	*F*	F_{ST}	G_{ST}	*Nm*	*ta*	Reference
Isozymes	Natural populations, undisturbed, seedlings	7	3.90	100.0	0.403	0.436	0.076	0.026	0.025	11.1	0.92	Reis (1996), Reis et al. (2000)
	Natural populations, undisturbed, adults	8	3.40	100.0	0.467	0.452	-0.033	0.031	0.013	10.4	-	Reis (1996), Reis et al. (2000)
	Natural populations with history of perturbation – seedlings to adults	1	2.60	62.5	0.264	0.278	0.050	-	-	-	-	Conte et al. (2003)
	Natural undisturbed populations	2	3.05	-	0.368	0.398	0.075	-	0.018	-	1.00	Conte (2004)
	Disturbed populations	2	3.05	-	0.388	0.434	0.105	-	0.041	-	1.00	Conte (2004)
	Natural undisturbed populations	1	2.10	69.2	0.195	0.216	0.097	-	-	-	-	Silva (2004)
	Disturbed populations located in small fragments	7	2.20	67.8	0.239	0.242	0.012	0.040	-	6.0	0.98	Martins-Corder et al. (2009)
	Conserved populations - seedlings	4	3.05	-	0.378	0.416	0.092	-	0.023	-	-	Conte et al. (2008)
	Conserved populations - saplings	4	3.15	-	0.385	0.431	0.107	-	0.017	-	-	Conte et al. (2008)
	Conserved populations – adults	4	3.12	-	0.403	0.424	0.050	-	0.011	-	-	Conte et al. (2008)
	Forest fragment, small natural population	1	-	100.0	0.588	0.467	-0.265	-	-	-	-	Vieira et al. (2010)
Microsatellites	Natural undisturbed populations – seedlings, juveniles, adults	2	10.80	100.0	0.690	0.749	0.081	0.060	-	3.4	0.86	Gaiotto et al. (2003)
	Natural undisturbed population (continuous forest) - seedlings, adults	1	10.70	100.0	0.609	0.786	0.257	-	-	-	0.91	Seoane et al. (2005a, b)
	Disturbed populations (isolated by fragmentation) – seedlings, adults	1	11.30	100.0	0.590	0.743	0.338	-	-	-	0.94	Seoane et al. (2005a, b)
	Natural undisturbed populations	2	14.06	100.0	0.690	0.770	0.104	-	0.025	-	1.00	Conte et al. (2006)
	Disturbed populations	2	14.18	100.0	0.665	0.792	0.160	-	0.038	-	1.00	Conte et al. (2006)
	Conserved populations – seedlings	4	14.12	-	0.678	0.781	0.133	-	0.024	-	-	Conte et al. (2008)
	Conserved populations – saplings	4	14.56	-	0.709	0.785	0.096	-	0.021	-	-	Conte et al. (2008)
	Conserved populations – adults	4	14.72	-	0.699	0.781	0.105	-	0.028	-	-	Conte et al. (2008)
	Forest remnants (five landscapes with different forest coverages) surrounded by agriculture – seedlings	16	6.2 – 9.2*	-	0.524-0.702**	0.716-.864**	-	-	-	-	-	Carvalho et al. (2015)
AFLP	Diverse populations: disturbed and undisturbed	11	-	92.1	-	0.119[1]	-	0.426	-	-	-	Cardoso et al. (2000)

*Allelic richness was determined here, instead. ** Range of values, instead of the averages.

technologies should be applied to better understand the population genetic mechanisms of the remaining populations of *E. edulis* to continue the purposes of conservation, sustainable management and breeding of the species.

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