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Effects of management on the genetic structure of *Euterpe edulis* Mart. populations based on microsatellites

Efeitos do manejo na estrutura genética de populações de *Euterpe edulis* Mart. com base em marcadores microssatélites

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Resumo

Foram investigados os efeitos da exploração tradicional e do manejo tecnificado sobre os níveis de variabilidade e estrutura genética de populações de *Euterpe edulis* (Arecaceae), comparando-se duas populações não perturbadas com duas populações exploradas, no sul do Brasil. Em cada população foram examinadas três coortes de plantas, usando 10 locos microssatélites. Os valores médios de diversidade genética ($\hat{A}$; $\hat{H}_e$; $\hat{H}_o$) foram altos e similares entre as diferentes coortes dentro de populações. A análise da estrutura genética ($\hat{G}_{st}$; $\hat{R}_{st}$) revelou que mais de 95% da variabilidade genética molecular se encontra distribuída dentro de populações. O processo de exploração, ocorrido nos últimos 40 anos, parece não ter causado alterações nos níveis de diversidade ou na estrutura genética das populações exploradas de *E. edulis*. Entretanto, valores mais expressivos de endogamia foram observados nas coortes mais jovens das duas populações exploradas. Sendo a ocorrência de cruzamentos não aleatórios relativamente comum em populações naturais de *E. edulis*, é possível que a redução do número de indivíduos reprodutivos nas populações exploradas tenha aumentado a frequência de cruzamentos entre indivíduos relacionados (parentes), uma vez que a autofecundação é um evento de ocorrência muito rara nesta espécie. Embora os efeitos tenham sido pequenos, a persistência do processo de exploração, especialmente o sistema tradicional, pode elevar ainda mais os níveis de endogamia e favorecer mudanças na estrutura genética das populações em gerações futuras.

Palavras-chave: Palmeira tropical, Exploração florestal, SSR, Palmiteiro, Variabilidade genética, Endogamia

Abstract

We investigated the effects of two exploitation systems – traditional and management - on the levels of variability and genetic structure of *Euterpe edulis* Mart. populations, by comparing two unlogged populations with two impacted populations in southern Brazil. Three cohorts, from seedlings to adults, were examined using 10 microsatellite loci. The mean values of genetic diversity ($\hat{A}$; $\hat{H}_e$; $\hat{H}_o$) were high and similar among the cohorts for the four populations. The estimates of interpopulation genetic variation ($\hat{G}_{st}$; $\hat{R}_{st}$) revealed that more than 95% of the molecular genetic variability was distributed within populations. Exploitation processes over the last 40 years appear not to have caused changes in the levels of variability or genetic structure of the disturbed populations of the species. However, higher inbreeding values were observed in the youngest cohorts of the two exploited populations. Regarding the common occurrence of non-random matings in natural populations of *E. edulis*, we believe that the reduction in the number of reproductive plants in exploited populations may have increased the frequency of matings among related individuals, since self-pollination is an event of rare occurrence in this species. Although the effects were small, the persistence of the exploitation process, especially the traditional system, can further elevate the inbreeding levels and favor changes in the genetic structure of the populations after successive generations.

Keywords: Tropical palm, Forest exploitation, SSR, Heart of palm, Genetic variability, Inbreeding

INTRODUCTION

Heart-of-palm tree (*Euterpe edulis* Mart.; Arecaceae) is a native shade-tolerant palm of the Brazilian Atlantic Forest. The heart of the palm is the edible apical meristem, considered to be one of the most important non-timber forest products exploited in the Atlantic Forest (CONTE *et al.*, 2003). Natural populations show a pyramid-shaped demographic structure, with a large base of juvenile plants and a small number of reproductive individuals (REIS *et al.*, 2000a).

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It is a monoecious species, with strongly protandrous flowers pollinated by insects (MANTOVANI and MORELLATO, 2000). Genetic studies using allozymes and microsatellite markers in natural populations have shown high values of outcrossing rates and gene flow for the species, together with high levels of genetic variability within populations and low interpopulation genetic divergence (REIS, 1996; REIS *et al*., 1998; CONTE *et al*., 2003; GAIOTTO *et al*., 2003).

However, currently natural populations are intensely fragmented, degraded and reduced in area of occurrence (REIS *et al*., 2000b). Although a sustainable management system has been proposed for the species (REIS *et al*., 1998; REIS *et al*., 2000a), the intensive exploitation occurring since the 1960's has resulted in the elimination of several populations and serious alteration of remaining populations (GALETTI and FERNANDEZ, 1998). The management system proposed for the species is based on three basic parameters: (1) population structure (DBH distribution); (2) increment rates; and (3) the number of reproductive trees per hectare (REIS *et al*., 2000a). Following these parameters, only individuals more than 9 cm DBH are harvested, with the maintenance of at least 50 reproductive individuals per hectare. On the other hand, the traditional system consists of harvesting most adult individuals, and few or no reproductive plants are left behind.

Although fragmentation and exploitation, such as selective logging, are common in tropical forests, there is a poor understanding of the effect of selective logging on the variability and genetic structure of the populations. Selective logging, commonly used for *E. edulis*, involves alterations in the size of populations as well as in the spatial distribution patterns of individuals within populations (BAWA and KRUGMAN, 1990; MURAWSKI, 1995). According to the exploitation intensity, such alterations can result in negative effects on the genetic structure of populations, such as loss of rare alleles, enhancement of inbreeding, and reduction of fitness of the subsequent generations (MURAWSKI *et al*., 1994; SEBBENN *et al*., 2000). Also, a strong reduction in population size can lead to random genetic drift, which can result in the fixation of alleles, and in increments of the relationship among individuals and inbreeding within populations (ELLSTRAND and ELAM, 1993; ALVAREZ-BUYLLA *et al*., 1996). In general, such consequences are more extreme in the youngest cohorts of remnant populations (MURAWSKI *et al*., 1994; ALDRICH *et al*., 1998; SEBBENN *et al*., 2000).

In this study, we investigated the effects of two exploitation systems - traditional and management - on the levels of variability and genetic structure of *Euterpe edulis* Mart. populations, using microsatellite markers. Our working hypothesis was that the exploitation process of the species, especially the traditional system, results in the loss of genetic variation and enhancement of inbreeding, therefore altering the genetic structure of its natural populations.

MATERIAL AND METHODS

Populations, sampling and DNA extraction

Four natural populations of *E. edulis* with different histories of disturbance were surveyed in the districts of São Pedro de Alcântara (SPA) and Ibirama (IB), Santa Catarina, Brazil (Table 1). At each site we chose an unlogged and an exploited population. Two exploitation systems were considered in the present study: (i) traditional (SPA), where most individuals higher than 2 m are harvested, including reproductive plants; and (ii) management (IB), where only individuals more than 9 cm DBH are harvested, with the maintenance of at least 50 reproductive individuals per hectare, according to the official regulations and policies for the management of natural populations of *E. edulis* for the State of Santa Catarina.

The vegetation of the study sites is defined as "Evergreen Atlantic Tropical Forest". In spite of the current fragmentation and degradation of the populations of *E. edulis*, many remnant fragments exist that are well conserved, as is the case of the two undisturbed populations sampled in this study. Also, the fragments still retain connectivity due to their mutual proximity, such that disturbed populations will benefit from the influence of undisturbed populations in the surrounding area. The disturbance history of the studied populations is quite different. In São Pedro de Alcântara, exploitation was carried out in different episodes between the 1960's and 1990's, a period in which regulations and policies presented no restrictions on heart of palm extraction. In Ibirama, although the population might have suffered exploitation in the past, the last management was carried out in 2000, following the regulations mentioned above. The geographical distance between the São Pedro and Ibirama sites is approximately 100 km. At Ibirama, the distance between the two populations is approximately 2 km, while in São Pedro it is 5 km.

Table 1. Characteristics of the four populations studied. (Características das quatro populações estudadas).

Populations	Locations	Adults.ha^{-1}	Altitude (m)	Latitude (S)	Longitude (W)
Pop. 1: Traditional	São Pedro	15	350	27º32'30''	48º47'22''
Pop. 2: Management	Ibirama	50	350	27º02'30''	49º27'27''
Pop. 3: Natural IB	Ibirama	130	350	27º02'25''	49º27'31''
Pop. 4: Natural SPA	São Pedro	120	300	27º32'15''	48º47'29''

To study variability and genetic structure, leaf material from three cohorts of plants was sampled randomly in the four populations as follows: i) Seedlings – insertion height of the youngest leaf less than 10 cm, corresponding to the individuals of the last reproductive period; ii) Saplings - insertion height from 30 cm to 1 m, absence of exposed stem and the presence of 4 to 5 mature leaves; and iii) Adults – plants usually higher than 10 m of height, presence of open inflorescence and/or abscission scars on the stem from previous events of reproduction. A total of 50 individuals were sampled per cohort in each population. Genomic DNA extraction from fresh leaf tissue followed standard CTAB procedure (DOYLE and DOYLE, 1987).

Microsatellite analysis

A group of 10 SSR loci, previously developed and optimized for *E. edulis* (GAIOTTO *et al.*, 2001) were used to genotype all sampled individuals. The total reaction volume used in PCR was 13 µl, containing 7.5 ng of genomic DNA; 50 mM KCl, 20 mM Tris-HCl pH 8.4; 10% dimethyl sulfoxide; 1.5 mM $MgCl_2$; 250 µM of each dNTPs; 0.3 µM of each primer (forward and reverse); 1.0 unit of *Taq* DNA polymerase (Ludwig Biotecnologia Ltda); and ultrapure water to complete the final volume. Amplifications were performed using a PTC-100 thermal controller (MJ Research) with the following conditions: 94°C for 5 min; 30 cycles of 94°C for 1 min, the primer specific annealing T_a temperature (GAIOTTO *et al.*, 2001) for 1 min, 72°C for 1 min; ending with 72°C for 7 min. The amplified fragments were separated in 4% polyacrylamide gel, in a run with 1x TBE at 60W for 1 h, using 10 bp ladder size standard, and stained with silver nitrate (CRESTE *et al.*, 2001).

Data analysis

Banding patterns were scored as genotypes and transformed into allele frequencies. These frequencies were subjected to a goodness-of-fit test (Fisher's exact test) to Hardy-Weinberg proportions, as defined by Weir (1996), using the GDA program (LEWIS and ZAYKIN, 2000). Such tests were performed by the conventional Monte Carlo method using 10 batches with 1,000 permutations per batch.

Molecular genetic variability was described in terms of average number of alleles per locus (A), observed heterozygosity (H_o), Nei's (1978) expected gene diversity (H_e), and fixation index (f), using the GDA program (LEWIS and ZAYKIN, 2000). The fixation index (f or F_{IS}) was estimated for each population by analysis of variance, and the significance was tested by bootstrapping over loci using 10,000 replicates in order to estimate interlocus variation.

Molecular genetic structure among populations was investigated by the analysis of gene diversity in subdivided populations (NEI, 1973). Parameters H_S, H_T, D_{ST} and G_{ST} were estimated using the FSTAT program (GOUDET, 2001), and the significance was tested by bootstrapping over loci using 10,000 replicates. The genetic distance among populations was estimated by Nei's unbiased genetic distances (NEI, 1978). A UPGMA cluster analysis was performed on the matrix of genetic distances using the GDA program (LEWIS and ZAYKIN, 2000).

As most mutations within microsatellites involve the addition or subtraction of a small number of repeat units, the mutation process is not in line with expectations under an infinite allele model with low mutation rates. Therefore, an analogue of the F_{ST} statistics, namely the R_{ST} parameter (SLATKIN, 1995), developed for microsatellite data, was also used to quantify genetic structure. Analysis of variance of allele size followed Goodman (1997), using the FSTAT program (GOUDET, 2001).

RESULTS

Genetic variation within populations

All 10 microsatellite loci displayed high levels of polymorphism in the four populations, with the least variable locus (EE5) and the most variable locus (EE47) displaying, respectively, 13 and 26 alleles. An average of 52 individuals was analyzed per cohort in each population, and a total of 161 alleles were detected. The mean values of diversity were high and homogeneous among the cohorts. Table 2 shows that the average number of alleles per locus ranged from

14.1 to 14.7, gene diversity ranged from 0.781 to 0.785, and the observed heterozygosity from 0.678 to 0.709. On a population level, the average number of alleles per locus of the three cohorts of plants was quite homogeneous among populations 1, 2 and 3, with a small decrease in Population 4 (Natural SPA). A similar trend was observed for gene diversity, since its magnitude depends on the number of alleles in each population. The observed heterozygosity was lower than gene diversity in all populations, indicating an excess of homozygotes. However, with the exception of Population 4 (Natural SPA, adults), the $\hat{H}_o$ values were slightly higher in the undisturbed populations.

The difference between estimates of H_e and H_o is applied to estimate inbreeding within populations ($\hat{f}$, Table 2). On average, seedlings showed a significant $\hat{f}$ value (0.133), while saplings and adults exhibited non-significant values (0.096 and 0.105, respectively, based on $CI_{95\%}$). However, regarding populations individually, some showed high and significant $\hat{f}$ values in the three studied cohorts. For seedlings, populations 1, 2 and 3 showed significant $\hat{f}$ values, but the most extreme values were found in the two exploited populations (1 and 2). For saplings, Population 2 also showed a significant $\hat{f}$ value. Although no difference was observed between the inbreeding coefficient at the seedling stage between disturbed and undisturbed populations based on confidence interval, there was an increased tendency in the estimates of fixation index in exploited populations.

Population genetic structure

The analysis of gene diversity in subdivided populations (Nei, 1973) revealed low genetic divergence among populations ($\hat{G}_{ST}$ = 0.024, 0.021 and 0.028 for seedlings, saplings and adults, respectively). A pairwise analysis displayed higher values of divergence between populations from different regions and lower values within regions. All estimates of G_{ST}, although low, were significantly different from zero when a confidence interval of 95% was used. The estimates of R_{ST} over all populations ranged from 0.033 to 0.048 among the three cohorts. In the pairwise comparisons, these estimates ranged from 0.004 to 0.091, and mostly were significantly different from zero. Despite the fact that the estimates of R_{ST} were higher than those of G_{ST}, results of both statistics revealed a close agreement, indicating that divergence was mostly among regions.

Nei's unbiased pairwise genetic distance between populations ranged from 0.053 to 0.146 for seedlings, 0.046 to 0.105 for saplings, and 0.063 to 0.154 for adults (Figure 1). The cophenetic correlation of the UPGMA clustering was high (0.750, 0.820 and 0.823 from seedlings to adults, respectively). All three cohorts displayed two distinct groups, the first composed of populations from Ibirama (2 and 3) and the second composed of populations from São Pedro de Alcântara (1 and 4). These results suggest that the exploitation process over the last 40 years had little influence on the pattern of molecular structuring, since the highest divergence levels were found among populations of different regions.

Table 2. Estimates of genetic parameters for seedlings, saplings and adults in four populations of *Euterpe edulis* based on 10 microsatellite loci. (Estimativas de parâmetros genéticos para plântulas, jovens e adultos em quatro populações de *Euterpe edulis* com base em 10 locos microssatélites).

Cohorts/Populations	N[1]	$\hat{A}$[2]	$\hat{H}_e$[3]	$\hat{H}_o$[4]	$\hat{f}$[5]	C.I.$_{95\%}$[6]
Seedlings						
Pop. 1: Traditional	53.3	14.1	0.798	0.668	0.163	0.011 to 0.337
Pop. 2: Management	51.1	14.2	0.787	0.662	0.159	0.035 to 0.262
Pop. 3: Natural IB	52.4	14.3	0.779	0.685	0.121	0.019 to 0.197
Pop. 4: Natural SPA	52.5	13.7	0.760	0.695	0.085 ns	-0.040 to 0.242
Average	52.3	14.1	0.781	0.678	0.133	0.013 to 0.254
Saplings						
Pop. 1: Traditional	50.7	14.0	0.797	0.722	0.095 ns	-0.008 to 0.206
Pop. 2: Management	52.1	15.2	0.792	0.644	0.188	0.015 to 0.261
Pop. 3: Natural IB	52.5	15.0	0.787	0.742	0.057 ns	-0.039 to 0.162
Pop. 4: Natural SPA	53.1	14.0	0.761	0.730	0.041 ns	-0.089 to 0.168
Average	52.1	14.5	0.785	0.709	0.096 ns	-0.010 to 0.211
Adults						
Pop. 1: Traditional	47.8	15.1	0.794	0.698	0.121 ns	0.000 to 0.230
Pop. 2: Management	50.2	14.8	0.789	0.698	0.116 ns	-0.043 to 0.300
Pop. 3: Natural IB	53.0	15.0	0.781	0.712	0.088 ns	-0.008 to 0.184
Pop. 4: Natural SPA	53.4	13.8	0.760	0.687	0.096 ns	-0.028 to 0.224
Average	51.1	14.7	0.781	0.699	0.105 ns	-0.007 to 0.222

[1]N, mean sample size per locus; [2]$\hat{A}$ average number of alleles per locus; [3]$\hat{H}_e$ expected heterozygosity; [4]$\hat{H}_o$ observed heterozygosity; [5]$\hat{f}$ fixation index; [6]C.I., nominal confidence interval (95%) obtained by bootstrapping over loci (10,000 replicates); [ns] non-significant.
[1]N, tamanho médio da amostra por loco; [2]$\hat{A}$ número médio de alelos por loco; [3]$\hat{H}_e$ heterozigosidade esperada; [4]$\hat{H}_o$ heterozigosidade observada; [5]$\hat{f}$ índice de fixação; [6]C.I., intervalo de confiança (95%) obtido por reamostragem sobre locos (10.000 bootstraps); [ns] não significativo.

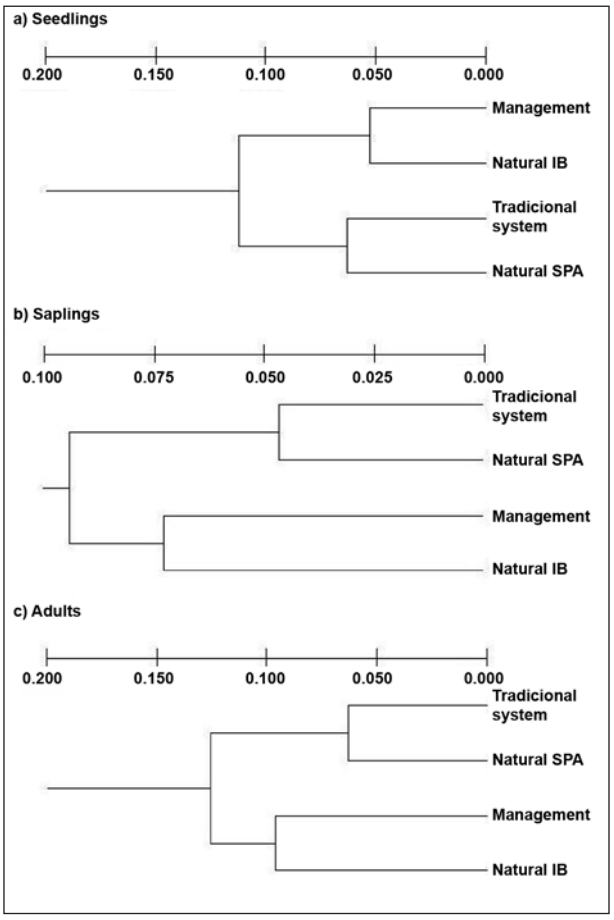

Figure 1. UPGMA dendrograms of four populations of *E. edulis* for seedlings (a), saplings (b), and adults (c) using Nei's unbiased genetic distance (NEI, 1978). (Dendrogramas UPGMA de quatro populações de *E. edulis* para plântulas (a), jovens (b), e adultos (c), usando a distância genética não viesada de Nei (NEI, 1978)).

DISCUSSION

The 10 microsatellite loci used in this work detected high levels of molecular genetic variation, confirming the high content of genetic information of these markers for studies of population genetic properties of *E. edulis* (GAIOTTO *et al*., 2003).

An examination of the estimates of genetic parameters, such as the number of alleles per locus and gene diversity, revealed that undisturbed and exploited populations exhibited similar levels of diversity for the three age cohorts (seedlings, saplings, and adults). As described by Crow and Kimura (1970), the maintenance of the heterozygosity levels over successive generations, in the absence of gene flow, depends on effective population size (N_e), the number of past generations (t), and the initial heterozygosity (H_o), where $H_t = (1–1/2N_e)^t H_o$. Therefore, a significant change in heterozygosity over a few generations is only possible if effective population size is drastically reduced, otherwise few effects will be observed. Thus, considering that the effective size of the two exploited populations is still relatively high, especially in the case of management (Population 2), and that the number of generations after exploitation is small (2-3 generations – 40 years), the level of heterozygosity at the time of the study does not yet appear to have been substantially influenced by the exploitation process.

In a test on the effects of genetic drift in remnant populations of *Acer saccharum*, Young *et al*. (1993) compared the levels of allozyme variation among eight remnant populations and eight similarly-sized samples from large intact populations of the species. Remnant populations as small as 96 trees showed no signs of reduced genetic variation, suggesting that there had been little effect of increased genetic drift during the 150-200 years (2-3 generations) since their formation by forest fragmentation. On the other hand, in exploited populations of *Tabebuia cassinoides*, Sebbenn *et al*. (2000) observed losses of alleles at low frequencies together with a significant reduction in the observed heterozygosity and gene diversity in the first generations after the exploitation. According to Young *et al*. (1996), the losses of genetic variation are more probably due to the formation of genetic bottlenecks at the time of exploitation and the subsequent inbreeding in small populations than to the effects of continuing random genetic drift.

However, interpopulation gene flow can play a significant role in maintaining genetic variability in exploited populations (YOUNG *et al*., 1996; HALL *et al*., 1996). Prober and Brown (1994) observed some effects of population isolation on genetic variation. Small remnant populations (<500 reproductive individuals) of *Eucalyptus albens*, located less than 250 m from a larger population, exhibited no obvious reductions in allelic richness, while more isolated remnants of a similar size, suffered strong genetic erosion. As demonstrated by Gaiotto *et al*. (2003), gene flow in *E. edulis* can occur over very long distances. In the present study, the two exploited populations are immediately surrounded by undisturbed populations, thus suggesting the occurrence of an extensive gene flow among them. Nevertheless, it is also possible that only after several successive generations of impact will variability levels begin to decrease, mainly in the traditionally exploited populations, where the effective population size of adult individuals is considerably reduced.

The estimates of interpopulation genetic var-

iation revealed that more than 95% of the molecular genetic variability of the species is distributed within populations. These results were similar to those of previous studies for this species (REIS, 1996; GAIOTTO *et al*., 2003). Moreover, our results are in agreement with other studies on tropical species, where species with large geographic ranges, outcrossing breeding systems and high rates of gene flow have more genetic diversity within populations and consequently a low divergence among populations (HAMRICK and GODT, 1990). The low differentiation among undisturbed and exploited populations indicates that the human intervention, at least at the time of study, has not caused changes in the genetic structure of populations. The greatest divergence was found between populations from different regions, mainly between the two undisturbed ones, and thus reflects pre-existing genetic structure. This structure suggests that natural *E. edulis* populations are differentiated by 'isolation by distance' (WRIGHT, 1943), with higher levels of gene flow among proximate populations, with gene flow decreasing as a function of distance. According to Reis *et al*. (1998), the continuous and abundant distribution of the species, originally in the whole area of the Atlantic Forest domain, tends to provide support for this model.

The estimates of the inbreeding coefficient ($\hat{f}$) displayed a considerable variation among loci within populations and were higher than those recorded in previous studies of this species (REIS *et al*., 1998; GAIOTTO *et al*., 2003). The finite size condition of a population has been considered as one of the causes that may explain the variation among estimated *f* values of different loci. Coelho and Vencovsky (2003) verified by simulation analysis that for populations with finite size, assuming linkage equilibrium, the inbreeding coefficient oscillated independently among loci over time. Thus, for populations with low effective size, samples of individuals in a single generation could result in high variation among estimated *f* values over different loci. On the other hand, the *f* values for some loci may have been overestimated due to statistical sampling (WEIR, 1996; COLLEVATTI *et al*., 2001). Because of the hypervariability of microsatellite loci used in this work, the number of genotyped individuals per population (~50) is relatively limited compared to the number of genotypes at a locus, and thus inbreeding may be overestimated.

Any way, inbreeding levels detected by the fixation index ($\hat{f}$) tend to be higher in exploited populations, especially in the youngest cohort. The possible effects of a reduction in the number of reproductive individuals in natural populations include an increase of selfing and mating among relatives, leading to increased inbreeding in future generations (BAWA and KRUGMAN, 1990; MURAWSKI, 1995; ALDRICH *et al*., 1998). SEBBENN *et al*. (2000) found an apparent increase in the inbreeding levels for exploited *Tabebuia cassinoides* populations in Brazil caused by changes in the reproductive behavior of trees, with an increment of the rate of self-pollination. Similar results were obtained by MURAWSKI *et al*. (1994), comparing undisturbed and exploited populations of *Shorea megistophylla* in Sri Lanka. However, the reproductive biology of *E. edulis* makes the occurrence of self-pollination difficult because the inflorescence is strongly protandrous, where male and female flowers open at different times (MANTOVANI and MORELLATO, 2000). In fact, the species displays a predominantly outcrossed mating system (REIS *et al*., 1998), with a high proportion of full-sibs within open-pollinated families (GAIOTTO *et al*., 2003). Although mating among relatives individuals is not a common in undisturbed *E. edulis* populations (GAIOTTO *et al*., 2003), selective logging probably has changed the behavior of pollinators, increasing non-random and mating among relatives (ALDRICH and HAMRICK, 1998; DAYANANDAN *et al*., 1999). Therefore, the increased inbreeding among the youngest individuals of the two exploited populations may have been caused by changes in the reproductive behavior. Despite the similarity between the two exploitation systems, the inbreeding of the saplings from the managed population (Ibirama) can be a reflex of other exploitation events which have occurred in the past, since these plants originated from reproductive events that took place prior to the implementation of management. Similar explanation may also be used for the inbreeding level observed among the seedlings of this population.

CONCLUSION

The exploitation process, at the time of the study, has not yet caused changes in the levels of variability and genetic structure of the *E. edulis* populations here investigated. However, higher inbreeding coefficients were observed in the

youngest cohorts of the two exploited populations possibly related to the reduction in the size of the mating population. Although the effects were small, the persistence of the exploitation process, especially the traditional system, can further elevate the inbreeding levels. After successive generations, such effects can lead to inbreeding depression and genetic erosion, and change the genetic structure of populations. However, our results pointed to additional questions to be studied. Because of the hypervariability of the microsatellite loci used in this work, we would recommend increasing sample size (>100) in order to optimize the genetic information provided by these markers. Moreover, new investigations into the effects of management are necessary, since the results of this study may have been influenced by other exploitation events that have occurred in the past and/or by gene flow between these populations and other surrounding undisturbed populations of the species. Finally, taking into account the tendencies observed in this study, we recommend the monitoring of critical areas of exploitation in future generations.

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