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PRIMER NOTE

Microsatellite markers for heart of palm – *Euterpe edulis* and *E. oleracea* Mart. (Arecaceae)

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Abstract

***Euterpe edulis* is the species of palm found in the Atlantic Forest and Cerrado gallery forest that yields the best heart of palm. A battery of 18 highly polymorphic microsatellite markers was developed from an enriched genomic library. Using fluorescence automated detection an average of 10.6 alleles per locus were found on a sample of 66 individuals sampled from a natural population. These loci allow extremely precise paternity testing, estimation of gene flow and of parentage coefficients among trees in the wild.**

Keywords: *Euterpe*, microsatellite, tropical tree

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Euterpe edulis is the species of palm that yields the best tasting heart of palm, the soft mass of nascent white tissues in the palm's stem, which is a highly appreciated food. This species, found throughout the Atlantic Forest as well as in gallery forests of Cerrado in Brazil, is currently seriously threatened due to the intensive and unsustainable exploitation of the existing populations in the Atlantic forest (Orlande *et al*. 1996).

We are interested in conservation and prebreeding strategies of valuable trees in the tropics such as the *Euterpe* species. As part of our project to estimate heritability for growth traits of *Euterpe* in the field, we developed a large battery of microsatellite markers to allow precise estimation of parentage coefficients among trees in the wild (Lynch & Ritland 1999).

Microsatellite markers were developed from an enriched genomic library constructed with *Sau*3A digested DNA from a single individual of *Euterpe edulis* according to protocols previously described (Brondani *et al*. 1998; Collevatti *et al*. 1999). Plasmid DNA was sequenced using dye-terminator fluorescent chemistry and products detected on an ABI 377 instrument (Applied Biosystems, CA). Primers complementary to sequences flanking the microsatellites were designed using the software Primer (Lincoln *et al*. 1991).

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A total of 475 colonies were screened as positive for $(AG)_n$ repeats. Anchored polymerase chain reaction (PCR) indicated that 170 of these (35.8%) had a microsatellite with adequate size and position in the cloned insert. From the 170 positive clones sequenced, 64 (37.6%) were used for designing primer pairs. Following primer screening, 18 primer pairs (28.1%) were selected to be used for genetic analysis. To characterize these 18 microsatellite markers for genetic information content, DNA was extracted from 66 individuals of *E. edulis* sampled from two different populations in gallery forests close to Brasília, D.F.

The PCR cocktail (13 μL) contained 7.5 ng of genomic DNA, 250 μM of each dNTPs, 0.75 μM $MgCl_2$, 1× PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$ pH 8.3), 2.5 μg/μL BSA, 0.2 μM of each primer, the forward primer dye-labelled, and 1 U of *Taq* DNA polymerase (Gibco). Amplifications were performed using a MJ Research PTC-100 thermal controller using the following protocol: 96 °C for 2 min; 30 cycles of 94 °C for 1 min, the primer specific annealing temperature T_a (see Table 1) for 1 min, 72 °C for 1 min, ending with 72 °C for 7 min. Alleles were sized on an automated DNA sequencer (ABI 377-XL) using GENESCAN software (ABI) and Rox-500 Size Standard (ABI).

All microsatellite marker loci were highly polymorphic with a mean of 10.6 alleles per locus. Loci EE15, EE32, EE4, EE54 and EE59 did not conform to Hardy–Weinberg expectations possibly due to a sampling effect or the presence

Table 1 Information on the microsatellite marker loci of *Euterpe edulis*. Primer sequences are listed with annealing temperatures (T_a), number of alleles per locus (A), expected heterozygosity (H_E), observed heterozygosity (H_O), probability of genetic identity (I) and the paternity exclusion probability (Q) based on genotypes for 66 unrelated adult individuals

Locus	Repeat array	Primer sequences (5′–3′)	Allele size range (bp)	T_a (°C)	A	H_E	H_O	I	Q	GenBank accession no.
EE2*	$(AG)_{22}$	F: CCAAggACgCAATCTCAA R: AgCgAggCAgAACACgTA	82–110	62	12	0.81	0.73	0.06	0.66	AF328879
EE3*	$(AG)_{11}.(AG)_{16}$	F: TTCgCgCACACTgAgAg R: ggTAgCgTTgATTggTCC	194–210	56	8	0.42	0.42	0.35	0.26	AF328881
EE5	$(AG)_{24}$	F: gAgAACACATAAgCTgC R: gCTTCAgAATTAggACA	102–136	56	13	0.82	0.75	0.06	0.65	AF328882
EE8*	$(AG)_{20}$	F: gTATTCCAATgTgCTCACAg R: gTgCAgTAggCTTCTAgTACC	110–132	58	8	0.74	0.75	0.11	0.51	AF328872
EE9	$(AG)_{19}$	F: TTCTCTCgCATgCCTCg R: gCCACACACACACAgTAgAATC	90–110	62	2	0.50	0.94	0.38	0.19	AF328880
EE15*	$(AG)_{21}$	F: CCACACAgACACgCAgATAg R: CCTCATgAAgCATCgACCT	140–162	64	12	0.82	0.98	0.06	0.64	AF328873
EE23*	$(A)_{14}(AG)_{23}$	F: gTTCTgCgATTCATACTCCTg R: TACgAACCAAgATggAgCAA	100–132	58	13	0.85	0.78	0.04	0.70	AF328877
EE25	$(AG)_{26}$	F: CggATCCTgAgACTgAATTg R: CCACACAgATTCAgAgCACA	156–190	58	9	0.82	0.73	0.06	0.63	AF328878
EE32	$(AG)_{20}$	F: CCgCCTggTgAgCCTCT R: CAgTgCACCAAggAACTCCAT	204–234	62	10	0.78	0.48	0.08	0.59	AF328883
EE41	$(AG)_{21}(TG)_{13}$	F: CCTTgCAgTTTATggCTACg R: CCATTgAgAgggAATgAggT	170–190	48	6	0.76	0.55	0.10	0.54	AF328884
EE43*	$(AG)_{16}$	F: gCgAAAggCTAACAACgTTAT R: AgCgAACCAACCAAgAAgAC	118–132	56	5	0.65	0.70	0.19	0.38	AF328886
EE45	$(AG)_{28}$	F: AAAgAAATTggCgTgACATC R: AACCAgTCTTCTCCCTCTCg	70–154	56	14	0.79	0.73	0.06	0.62	AF328887
EE47	$(AG)_{20}$	F: CgAAATCAATggTTTCAgTg R: AATTATTgTTgTgggCAgC	214–246	56	11	0.84	0.80	0.05	0.67	AF328874
EE48	$(AG)_{27}$	F: CCTACCATTACgTACTgTCg R: CAATATCAAgCTCATCCATC	210–266	56	19	0.88	0.59	0.03	0.76	AF328875
EE52	$(AG)_{22}$	F: TTCTgTggAgAgTCAATCATC R: AATCTgACAAggCCTCAAC	230–260	56	14	0.87	0.74	0.03	0.74	AF328888
EE54*	$(AG)_{25}$	F: CATgTATCTAAggAACAAgg R: CTgTgCTCTCTCATTCTCA	140–160	56	9	0.77	0.42	0.09	0.56	AF328876
EE59	$(AG)_{16}$	F: AACCTCTCTTTggCCTA R: CTTggCATACTggAACC	84–128	56	12	0.81	0.61	0.06	0.63	AF328885
EE63	$(AG)_{18}$	F: CCgATATgCTCAAATCAATg R: ACgAgAggAATCAAAgAACC	106–132	56	15	0.88	0.74	0.03	0.75	AF328889

*Locus successfully transferred to *Euterpe oleracea*.

of null alleles. Parameters of genetic information content were estimated, including the probability of genetic identity (I) (Paetkau *et al*. 1995), which corresponds to the probability of two random individuals displaying the same genotype and the paternity exclusion probability (Q) (Weir 1996), which corresponds to the power with which a locus excludes an individual tree of being the parent of an offspring (Table 1). Independence amongst all 18 loci could not be assumed as a number of two-locus linkage disequilibrium tests were deemed significant. However, smaller batteries of six markers all in Hardy–Weinberg equilibrium and linkage equilibrium could be easily defined (e.g. EE2, EE3, EE8, EE23, EE43 and EE45) that combined reached multilocus estimates of Q and I of 0.991 and 1.05×10^{-6}, respectively. Out of 18 microsatellite loci developed for *E. edulis*, seven could successfully amplify clearly interpretable hypervariable products in *E. oleracea*, another important species endemic to the Amazon forest (Table 1). The 18 microsatellite marker loci have allowed very precise inferences of pairwise relatedness amongst individuals in natural populations. Relatedness measures were in turn used to estimate heritability for juvenile growth traits aiming at the selection of a base population for the establishment of a breeding programme for *E. edulis*.

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