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PRIMER NOTE

Microsatellite markers for *Ceiba pentandra* (Bombacaceae), an endangered tree species of the Amazon forest

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Abstract

From a genomic library enriched for AG/TC repeats, eight polymorphic microsatellite markers were developed for *Ceiba pentandra*, a pan-tropical forest tree. Polymorphism was evaluated using a panel of 74 adult trees. Using automated fluorescence detection, a total of 112 alleles was detected with an average of 14 alleles per locus. All microsatellite loci showed very high levels of genetic information content, with expected heterozygosity ranging from 0.814 to 0.895. These microsatellite markers represent a powerful tool to investigate refined questions of mating systems, gene flow, family structure and population dynamics in natural populations of *C. pentandra*.

Keywords: Bombacaceae, *Ceiba pentandra*, microsatellite, tropical tree

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Ceiba pentandra (Bombacaceae), the kapok tree, is an emergent, fast-growing tree species with pan-tropical distribution. The species grows naturally in tropical America and Africa, whereas populations from Southeast Asia were probably introduced by man (Baker 1965). In the Amazon Basin, this giant tree occurs in the lowland, seasonally-flooded 'várzea' forest. Native populations of *C. pentandra* in the Peruvian and Brazilian Amazon are threatened because of intensive exploitation by the plywood industry.

The development of microsatellite markers provides a valuable tool for rapidly generating information on the patterns of genetic variation, gene flow and mating systems in natural populations to develop better strategies for sustainable management and conservation of tropical tree species. Here, we report the development and characterization of a highly informative battery of eight microsatellite markers for *C. pentandra*.

An enriched genomic library from a single *C. pentandra* tree was constructed with *Tsp 509* digested DNA, following protocols described by Brondani *et al.* (1998). Recombinant clones in plasmid vectors were transformed into *E. coli* cells according to Collevatti *et al.* (1999), and colonies having simple sequence repeat (SSR) were identified by poly AG/TC probe hybridization. Positive clones were picked and sequenced using dye-terminator fluorescent chemistry, and resolved on an ABI 377 instrument. Primers to the SSR flanking regions were designed using the software PRIMER (Lincoln *et al.* 1991).

Out of 300 recombinant colonies, 49 (16%), identified as positive for AG repetitive sequence, were sequenced. As expected, AG repeats were confirmed for most of them. However, due to the complexity of the AG repeats inside the clone, primers flanking the SSR region were designed for 22 of them. Following microsatellite marker screening by silver nitrate detection in polyacrylamide gels, eight loci were selected based on a higher level of allelic variation, low stutter and robustness of interpretation in a sample of six individual plants.

To carry out detailed genetic analysis, forward primers labelled with HEX, 6-FAM and NED fluorescent dyes were synthesized to allow multiplexed electrophoresis and allele scoring. PCR amplification was carried out in a 13 μL volume containing 7.5 ng genomic DNA, and 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$ (pH 8.3), 0.2 mM of each

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Table 1 Information on the eight microsatellite marker loci of *Ceiba pentandra*. Annealing temperature (T_a), total number of alleles (A), expected heterozygosity (H_E), observed heterozygosity (H_O), probability of genetic identity (I) and paternity exclusion probability (Q)

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	T_a	A	H_E	H_O	I	Q	GenBank accession no.
CP1	ggACTCTAggCTCTgCTCTACT gTggAAggTgCACAAggA	$(AG)_{27}$	182–236	65	17	0.845	0.360	0.040	0.711	AF503157
CP12	CTgTgAgTgTCAggATgCTT TgAACgTgAACATgTgTgTg	$(CT)_{19}$ $(CA)_{30}$	81–133	53	17	0.871	0.777	0.027	0.758	AF503158
CP13	ACCACgATTCCgTTCAgg CTggCAgTCgTTCTgTTgAg	$(AG)_{16}$	105–125	65	11	0.834	0.667	0.044	0.695	AF503159
CP15	CCAgCAggTACTCAggTCACTT TgATCAggACATgAggCAgg	$(TC)_{19}$	91–109	65	10	0.814	0.690	0.058	0.655	AF503160
CP18	gATTgTCTCCTCggCTCAT gATCAggCTCCAAgTTCTCT	$(CTT)_3$ $(GA)_{25}$	117–153	65	15	0.873	0.592	0.027	0.760	AF503161
CP19	TgTgAggCAggTATggTTCT TTgATgCACggTgCATAATC	$(TC)_{19}$	117–141	69	12	0.833	0.810	0.043	0.698	AF503162
CP20	GCATATAgAgACgCTAgCTT GACAgTgCAgATTgCAgAg	$(CT)_{22}$	142–184	65	15	0.895	0.860	0.019	0.797	AF503163
CP22	TATTCTCAAgTCATggCCTC AACTgCgAACTgTgAAggA	$(GA)_{18}$	249–271	63	15	0.823	0.727	0.051	0.670	AF503164

dNTP, 4% dimethyl sulfoxide, 1 U of *Taq* polymerase and 0.2 μM of each primer (forward primer dye-labelled). The reactions were performed on a 96-well PT-100 Thermocycler (MJ Research) at 94 °C for 4 min, then 30 cycles at 94 °C for 1 min, 53 °C for 1 min (or 63, 65 and 69, depending on the locus), 72 °C for 1 min, and a final extension step at 72 °C for 7 min. The amplified products were separated in a 5% polyacrylamide gel on an automated DNA sequencer (ABI 377) and alleles sized by comparison with an internal sizing standard (Brondani & Grattapaglia 2001).

Numbers of alleles, expected and observed heterozygosities were estimated using the software Genetic Data Analysis (Lewis & Zaykin 1998) based on a sample of 74 adult trees located in the central Brazilian Amazon. Parameters of genetic information content were also estimated. These included 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), corresponding to the power with which a locus excludes a tree of being the parent of an offspring (Table 1). None of the eight loci conformed to Hardy–Weinberg expectations. The observed heterozygosity was lower than the expected heterozygosity, probably due to population substructuring in the sampled population as a result of selfing and/or related matings in the constitution of the current adult population. The number of different alleles per locus, however, was high, ranging from 10 to 17, with a mean of 14. As several two-locus linkage disequilibrium tests were deemed significant, estimates of I and Q using all eight loci with the product rule would not be valid. However, smaller batteries of four to five markers in linkage equilibrium could be defined which, combined, reached multilocus estimates of Q and I of 0.98 and 10^{-4}, respectively. In conclusion, the microsatellite markers developed allow very precise individual discrimination, paternity testing and mating system studies. We are currently using these markers to investigate the patterns of long distance gene flow in natural populations of *C. pentandra*.

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References

Baker HG (1965) The evolution of the cultivated kapok tree: a probable West African product. In: *Ecology and Economic Development in Tropical Africa* (ed. Brokensha D), pp. 185–216. Institute of International Studies, University of California, Berkeley.

Brondani RPV, Brondani C, Tarchini R, Grattapaglia D (1998) Development, characterization and mapping of microsatellite markers in *Eucalyptus grandis* and *E. urophylla*. *Theoretical Applied Genetics*, **97**, 816–827.

Brondani RPV, Grattapaglia D (2001) Cost-effective method to synthesize a fluorescent internal DNA standard for automated fragments sizing. *Biotechniques*, **31**, 793–800.

Collevatti RG, Brondani RPV, Grattapaglia D (1999) Development and characterization of microsatellite markers for genetic analysis of Brazilian endangered tree species *Caryocar brasiliense*. *Heredity*, **83**, 748–756.

Lewis P, Zaykin D (1998) *Genetic Data Analysis: Computer Program for the Analysis of Allelic Data, Version 1.0 (d12)*. http://chee.unm.edu/gda/.

Lincoln SE, Daly MJ, Lander ES (1991) *'Primer' Software*. *Available from* E. Lander, Whitehead Institute, Cambridge, MA, USA.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.

Weir BS (1996) *Genetic Data Analysis II*. Sinauer Associates, Sunderland, MA.