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PRIMER NOTE

Isolation and characterization of microsatellite loci in the tropical tree *Jacaranda copaia* (Bignoniaceae)

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Abstract

We isolated 10 microsatellite loci for *Jacaranda copaia* for direct measurement of seed and pollen movement within the 50 ha Forest Dynamics Plot on Barro Colorado Island, Panama, and for studies of population genetic structure and gene flow across the Isthmus of Panama. Enriched repeat libraries were screened for microsatellite repeats and polymorphic primer pairs were developed. A total of 110 adult individuals were screened for allelic diversity and loci showed high levels of heterozygosity. These loci show both high maternal and paternal exclusion probabilities.

Keywords: *Jacaranda copaia*, microsatellite, Panama, paternity and maternity analysis, tropical tree

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Direct studies of gene flow via seed in plants have been hampered by the lack of a straightforward method of identifying, unambiguously, the maternal parent in hermaphroditic species. However, this problem can be overcome by genotyping maternal tissue that usually surrounds seeds, including seed endocarp, seed wings or samaras (Godoy & Jordano 2001; Wang & Smith 2002). As seed coat tissue is maternal tissue and an exact diploid match to the maternal parent in the population, seed maternal parentage can be assigned to dispersed seeds with knowledge of adult genotypes in the population. With this goal in mind, we developed microsatellite markers to study directly seed and pollen movement of *Jacaranda copaia* within the 50 ha Forest Dynamics Plot (FDP) on Barro Colorado Island (BCI), Panama, and to investigate the population genetic structure of this species across the Isthmus of Panama.

Jacaranda copaia is a large, wind-dispersed pioneer tree that colonizes large tree-fall gaps (Brokaw 1985). It ranges from Bolivia to Belize and is a common pioneer within the FDP. Previous work has shown that the wind-dispersed seeds of *J. copaia* are among the most well-dispersed seeds within the FDP (Dalling *et al*. 2002). Mating system studies have shown that *J. copaia* is a predominantly outcrossed species (James *et al*. 1998).

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We used a hybridization selection method (Kandpal *et al*. 1994; Kijas *et al*. 1994) to create an enriched CA repeat library. Genomic DNA was restricted using *Sau3AI*, and size fractionation was carried out using ChromaSpin columns (Clontech Laboratories) to isolate fragments larger than 400 bp. *Sau3AI* linkers were ligated to the fragments, run through the ChromaSpin column to remove excess linker and amplified to create a whole genome polymerase chain reaction (PCR) library using the following PCR reaction: 1 × PCR buffer, 1.5 mM $MgCl_2$, 15 μL purified ligation reaction, 100 μM each dNTP, 0.5 μM *Sau*3AI primer and 2.5 U *Taq* polymerase (Sigma) in a total reaction volume of 100 μL. Cycle conditions consisted of 3 min at 94 °C, followed by 25 cycles for 1 min at 94 °C, 1 min at 68 °C, 2 min at 72 °C and a final elongation step of 10 min.

Selective hybridization was carried out using Vectrex Avidin D (Vector Laboratories) and a biotinylated probe [5′-$(CA)_{15}$TATAAGATA-Biotin]. A second PCR reaction was carried out after this step to increase the number of genomic fragments enriched for CA repeats. A 1 μL aliquot of the PCR product was ligated into TOPO TA plasmid vector (Invitrogen) and transformed into *Escherichia coli* according to the manufacturer's protocol.

Colony lifts were screened using the CA_{15} probe and the Phototope chemiluminescent detection system (New England Biolabs). Thirty colonies showing strong hybridization signals were sequenced using the BigDye Terminator Kit (Applied Biosystems) according to the manufacturer's

Table 1 Locus name, primer sequences, Annealing temperature (T) repeat motif, allele size range, number of individuals genotyped (*N*), number of alleles observed (*k*), observed (H_O) and expected (H_E) heterozygosity, and GenBank accession number

Locus	Primer sequences	T	Repeat	Size	*N*	*k*	H_O	H_E	F_{IS}	Accession no.
JACC 1	F: CGGCTCATGGGCAGTACC	63	$(CA)_{15}$	185–207	110	5	0.800	0.585	−0.370†	AY257038
	*R: GGCTTCATACCACTGATTGC									
JACC 2	F: ACGATAAATGGGCGAGAGAG	63	$(CT)_{19}(CA)_{10}$	207–233	110	15	0.836	0.819	−0.022	AY257039
	*R: GGTGACTTGGGTTGATGTTCTTT									
JACC 4	F: TCCCTAATCTTGCCTGCTTG	60	$(GT)_{14}(GA)_{8}$	207–267	110	24	0.764	0.916	+0.106†	AY257040
	*R: ACTCACTGGGCGCTCGAC									
JACC 11	F: GGACATGCACTTTCCTTTTATG	63	$(CA)_{16}$	145–153	110	5	0.636	0.660	+0.036	AY257041
	*R: TGATGGAGAATATCTAAAGCACTCC									
JACC 18	F: TGGGACATCTACAGCCAAGTC	63	$(GT)_2GA(GT)_{11}$	268–300	110	15	0.964	0.899	−0.072†	AY257042
	*R: AGACAATGAGAAACGCCACTC									
JACC 21	F: ATCTCCTCAAACACCCACAT	63	$(GT)_9$	230–250	110	10	0.745	0.567	−0.317†	AY257043
	*R: TCATTGCCTCCAACACACTT									
JACC 22	F: TTGACAGCTCTTTGTTTGAACC	58	$(GT)_{27}$	164–217	110	24	0.945	0.880	−0.075†	AY257044
	*R: AAGATTCCTTTTGACAGCTTCG									
JACC 1.1	F: CTCCTGACGAAGTCAAAAAG	54	$(GT)_{12}$	110–144	110	16	0.864	0.823	−0.050	AY257045
	*R: ATCAAGTTTCAGAAGACAGG									
JACC 9	F: AATAATTAACTCAACTGGCCACTCC	61	$(GT)_{15}$	173–197	110	11	0.936	0.844	−0.110†	AY257046
	*R: TTTAGTTGTCTCCCTGTTTGAGATG									
JACC 31	F: TCCTTAGCTTTGAAATGAATAAAAGAG	63	$(CA)_{16}$	176–194	110	10	0.918	0.792	−0.160†	AY257047
	*R: ACGTAGAGGGGATTTGTAATTTCTC									
Average						13.5		0.778		

*The fluorescently-labelled primer in the reaction.
†Significant departure from HW equilibrium ($P < 0.05$).

protocol with the T7 primer, and electrophoresed on an ABI 3700 capillary electrophoresis sequencer (Applied Biosystems). Sequences were aligned and contigs created using SEQUENCHER (GeneCodes).

Primers were designed for 15 repeat regions using PRIMER 3 web-based software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) and synthesized (IDT Technologies). Total genomic DNA was isolated from adult tissue using a standard cetyltrimethyl ammonium bromide (CTAB) method (Doyle & Doyle 1990), and resultant samples were further diluted 1 : 4 with deionized water. PCR reactions contained 1 μL template DNA, 1 × GeneAmp buffer II, 2.5 mM $MgCl_2$, 200 μM each dNTP, 125 μM each of forward and reverse primers, and 0.5 U AmpliTaq Gold DNA polymerase (Applied Biosystems) for a total volume of 10 μL per reaction. After experimentation with annealing temperatures, levels of polymorphism of all primer pairs were checked on a sample of 10 randomly-selected adult individuals by adding 0.01 μL fluorescently-labelled [F]dCTP (Applied Biosystems) to the PCR cocktail. Cycling conditions consisted of 7 min at 95 °C, followed by 30 s at 95 °C, 30 s at annealing temperature (Table 1), a 72 °C extension for 30 s and a final extension at 72 °C for 5 min on an Eppendorf Master Cycler (Eppendorf).

For the 10 loci that showed allelic variation, fluorescently-labelled primers (6-FAM, HEX: IDT Technolgies; and NED: Applied Biosystems) were synthesized. Genotypic data were collected from 110 randomly-selected adult individuals from the FDP population. After amplification, 1 μL PCR product was mixed with 1.1 μL loading solution (0.7 μL deionized formamide, 0.25 μL ROX and 0.15 μL loading dye; Applied Biosystems). Samples were electrophoresed on 4.75% acrylamide gels and an ABI 377 using GENESCAN. These markers can also be successfully amplified from maternal tissue taken from seed wings of this species.

Allele frequencies were examined first in CERVUS 2.0 (Marshall *et al*. 1998) (Table 1). Deviations from Hardy–Weinberg equilibrium were further explored in GENEPOP version 3.3 (http://wbiomed.curtin.edu.au/genepop/ Raymond & Rousset 1995) using the probability test with default values. F_{IS} values are reported according to the method of Weir & Cockerham (1984). Loci showed high levels of observed and expected heterozygosity. Allelic richness ranged from five to 24 alleles per locus with an average of 13.5 alleles per locus. The expected heterozygosity ranged between 0.567 and 0.916 and the average expected heterozygosity was 0.778. Loci JACC4 and JACC11 showed significant deviations from Hardy–Weinberg equilibrium, specifically, heterozygote deficit consistent with the presence of null alleles. All loci yielded high exclusion probabilities. For example, collectively, these loci showed total exclusionary power of the first parent to

be 0.998. These markers will be of great use in direct measurement of the movement of seeds and pollen within this population, and in estimating population structure across the Isthmus of Panama and elsewhere in the tropics.

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