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PRIMER NOTE

Eight microsatellite markers for the neotropical tree *Luehea seemannii* (Tiliaceae)

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Abstract

We isolated eight polymorphic microsatellites from the neotropical tree *Luehea seemannii* for gene flow and genetic structure studies. We used a streptavidin subtractive enrichment technique to develop a library of CA/GT repeats. Eight loci were screened for diversity from 96 individuals from Barro Colorado Island (BCI) and neighbouring Gigante peninsula in Panama. *Luehea seemannii* shows moderate levels of genetic diversity within these two populations. Allelic richness ranged from four to nine alleles and averaged 6.44 alleles per locus. Average expected heterozygosity was 0.65 on BCI and 0.60 on Gigante. Results are compared to microsatellite data from another wind-dispersed gap colonizing species common in Panama.

Keywords: Barro Colorado Island, gene flow, maternity and paternity analysis, Panama, seed dispersal

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Nuclear microsatellite markers have been shown to be useful in studies of population genetic structure and gene flow on small and large scales (Chase *et al*. 1996; Zhang & Hewitt 2003). Direct studies of gene flow via seed and pollen within large natural populations require markers with a large number of alleles and with high heterozygosities to assign parentage unambiguously. As part of a larger effort to understand population genetic patterns on local and regional scales in tropical trees, we isolated and characterized eight microsatellite loci in *Luehea seemannii* for use in direct studies of seed and pollen movement within a large mapped tropical forest census plot on Barro Colorado Island (BCI), Panama and for surveys of genetic diversity from populations across the Isthmus of Panama.

Luehea seemannii Tr. & Planch. (Tiliaceae) is a large, light-demanding canopy tree, common in young forest and roadsides in the Panama Canal watershed (Croat 1978) and in tree fall gaps in old growth forest (Dalling *et al*. 2002). It is a characteristic tree of tropical moist forest throughout Panama and from dry and wet forests in Costa Rica. It produces small white and yellow flowers that are pollinated by a large variety of insects (Haber & Frankie 1982) and many small (6–10 mm long) winged seeds in five-lobed oblong capsules (Croat 1978). A long-term study of seed rain on BCI has shown *L. seemannii* to be among the most widely dispersed gap colonist species within the forest (Dalling *et al*. 2002). Bawa *et al*. (1985) list *L. seemannii* as self-incompatible, whereas Haber & Frankie (1982) show it to be partially to fully self-compatible, in response, perhaps, to lower pollinator abundance due to lower levels of rainfall at the edge of its range in Costa Rica.

We used a hybridization selection method (Kandpal *et al*. 1994; Kijas *et al*. 1994; Jones & Hubbell 2003) to create an enriched CA repeat library for *L. seemannii*. Genomic DNA was extracted from adult leaves using a standard cetyltrimethyl ammonium bromide (CTAB) extraction method (Doyle & Doyle 1990). Genomic DNA was restricted using *Sau*3AI enzymes and size fractionation was done using ChromaSpin columns (Clontech Laboratories) to isolate fragments larger than 400 bp. *Sau*3AI linkers were ligated to the fragments and again run through the ChromaSpin column to remove excess linkers. Fragments with linkers were then used to create a whole genome polymerase chain reaction (PCR) library using the following reaction: 1 × PCR buffer, 1.5 mM $MgCl_2$, 15 μL of purified ligation reaction, 100 μM each dNTP, 0.5 μM *Sau*3AI primer, and 2.5 units of *Taq* polymerase (Sigma) in a total reaction vol. of 100 μL. Cycle conditions consisted of 3 min at 94 °C, followed by 25 cycles for 1 min at 94 °C,

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Table 1 Locus name, primer sequences, optimal annealing temperature (T), repeat motif, allele size range, number of individuals genotyped (n), number of alleles observed (k), observed heterozygosity (H_O), expected heterozygosity (H_E), and F-statistics for Barro Colorado Island (BCI) and Gigante peninsula (GIG) populations, and GenBank Accession number for eight *Luehea seemannii* microsatellite loci

Locus	Primer sequences	T	Repeat	Size	n	k	H_O BCI	H_E BCI	F_{IS} BCI	H_O GIG	H_E GIG	F_{IS} GIG	F_{IS}	F_{ST}	F_{IT}	Accession number
LUES2	F: TCCATAGCAACCCTACCTGTG R: CTTCTGCGCCATGTGATTC*	61	$(GA)_2(GT)_{23}(GA)_{20}$	169–175	82	4	0.254	0.677	0.624†	0.435	0.657	0.036	0.546†	–0.019	0.538	AY376383
LEUS3	F: GTGTCATGCTCTGTCTGTGC R: GTGGAAGAAAATGCCTCCTG*	61	$(GT)_{11}$	165–177	89	6	0.508	0.601	0.155	0.291	0.302	0.036	0.137	0.047	0.179	AY376384
LUES6	F: TCATTCATCGTCCCCAAAATTTAC R: ACCAATGCCCTCTTGACCAC*	61	$(GT)_{23}$	146–171	90	8	0.651	0.670	0.027	0.583	0.587	0.007	0.022	0.012	0.035	AY376385
LEUS7	F: GAGGACTGAGAAGCAGAAATGC R: GCTCCAACATCGGGAAAAAG*	61	$(GT)_{14}$	170–180	75	5	0.259	0.367	0.295†	0.428	0.343	–0.250	0.151†	–0.015	0.138	AY376386
LEUS11	F: GCTTAGTCCTAGCATTTTGC R: GGGTAGGCAGGTAGGAGACC*	61	$(GT)_{16}$	169–195	69	8	0.796	0.839	0.052	0.700	0.707	0.0093	0.042	0.035	0.076	AY376387
LEUS17	F: AACACAGCCTACTTTATCCAAC R: TAACAGGTATGGGTCCTTCC*	61	$(GT)_{15}(GC)_3(GTGC)_2(GT)_7(GA)_{10}$	185–209	91	9	0.700	0.700	0.001	0.722	0.786	0.081	0.023	0.071	0.093	AY376388
LEUS50	F: AAACAGCGACAATGGGTTTC R: CCGGTTTCTAAAAGTAGCTTGC*	61	$(GT)_{14}$	237–251	89	5	0.723	0.672	–0.075	0.625	0.549	–0.139	–0.089	0.017	–0.070	AY376389
LUES88	F: TGGAGATTTATTGCGTTTTGG R: AAATAGAGGGCGGCTGCTAC*	61	$(GT)_{14}$	183–211	66	5	0.160	0.599	0.732†	0.313	0.742	0.579†	0.688†	0.031	0.698	AY376390
Average						6.44	0.539	0.647	0.167	0.536	0.595	0.099	0.1485	0.0286	0.1729	

*Refers to the fluorescently labelled primer in the reaction.
†Denotes significant departure from Hardy–Weinberg equilibrium at $P < 0.01$.

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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]. The enriched library was then checked via serial dilution and chemiluminescent detection system using the above biotinylated probe to detect the success of the enrichment technique. A second PCR reaction was carried out on the enriched library to increase the copy number of fragments containing microsatellite repeats. One microlitre of this PCR product was then ligated into TOPO TA plasmid vector (Invitrogen) and transformed into *E. 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). Sixteen colonies that showed strong hybridization signals were sequenced using the BigDye Terminator Kit (Applied Biosystems) according to the manufacturer's 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 12 repeat regions using Primer3 web-based software (http://www-genome.wi.mit.edu/cgibin/primer/primer3_http://www.cgi) and synthesized (IDT Technologies). Total genomic DNA was isolated from adult tissue using a standard CTAB method (Doyle & Doyle 1990) and extractions were further diluted 1:5 with deionized water for PCR reactions. PCR reactions for annealing temperature and $MgCl_2$ optimization experiments and all subsequent genotyping reactions contained 1 μL of template DNA, 1 × GeneAmp buffer II, 3.0 mM of $MgCl_2$, 0.2 μM of each dNTP, 0.125 μM of forward and reverse primers each, and 0.5 units of AmpliTaq DNA polymerase (Applied Biosystems) for a total vol. of 10 μL per reaction. 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). Fluorescently labelled reverse primers (6-FAM, HEX, IDT Technologies, and NED, Applied Biosystems) were then synthesized for those primer pairs that amplified product within the range of the cloned repeat.

Genotypic data were collected from 66 adult individuals from the BCI 50-ha Forest Dynamics Plot and from 30 adults from a population on the neighbouring Gigante peninsula. After PCR amplification, 1 μL of pooled PCR product from up to three loci was combined with 5 μL of loading solution (4.75 μL of HiDi formamide and 0.25 μL of 400HD ROX size standard, Applied Biosystems). Samples were electrophoresed on an ABI 3700 capillary electrophoresis machine and loci were scored in GeneScan 3.7 and Genotyper 3.6 (Applied Biosystems).

Allele frequencies were examined first in CERVUS 2.0 (Marshall *et al*. 1998) Deviations from Hardy–Weinberg equilibrium and evidence of linkage disequilibrium among loci were further explored in GENEPOP (version 3.3, http://wbiomed.curtin.edu.au/genepop/; Raymond & Rousset 1995) using the probability test with default values (option 2 and 3). *F*-statistics are reported according to the method of Weir & Cockerham (1984). Allelic richness ranged from four alleles in locus LEUS2 to nine alleles in LEUS17 and averaged 6.44 alleles per locus (Table 1). Loci showed a large range of observed levels of heterozygosity (Table 1), ranging from 0.16 in locus LEUS88 on BCI to 0.796 in locus LEUS11 on BCI. The average expected heterozygosity from the BCI and Gigante populations was 0.647 and 0.595, respectively. The total exclusionary power across all loci in a parentage analysis was 0.932 for the first parent and 0.993 for the second parent. *F*-statistics show that three loci, LEUS2, LEUS7, and LEUS88 show significant departures from Hardy–Weinberg expected values within the BCI population and LEUS88 shows significant departure from HW on Gigante (Table 1). All departures from equilibrium indicate heterozygote deficit. Most genetic diversity appears to be partitioned within populations (Table 1). No evidence for linkage disequilibrium was found between loci at $P < 0.01$.

Luehea seemannii shows a lower average number of alleles per locus and less genetic diversity within the BCI population than does *Jacaranda copaia*, another gap-colonist from BCI with winged seeds (Jones & Hubbell 2003). Ten microsatellite loci isolated from *J. copaia* averaged twice the number of alleles per locus, as well as showed 25% greater observed heterozygosity than these eight *L. seemannii* loci. Regardless, these eight markers will be useful for studies of the movement of genes within the BCI population and in genetic diversity surveys in Panama and elsewhere.

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References

Bawa KS, Perry DR, Beach JH (1985) Reproductive biology of tropical lowland rain forest trees. I. Sexual systems and incompatibility mechanisms. *American Journal of Botany*, **72**, 331–345.

Chase M, Kesseli R, Bawa K (1996) Microsatellite markers for population and conservation genetics of tropical trees. *American Journal of Botany*, **83**, 51–57.

Croat TB (1978) *Flora of Barro Colorado Island*. Stanford University Press, Stanford, California.

Dalling JW, Muller-Landau HC, Wright SJ, Hubbell SP (2002) Role of dispersal in the recruitment limitation of neotropical pioneer species. *Journal of Ecology*, **90**, 714–727.

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus*, **12**, 13–15.

Haber WA, Frankie W (1982) Pollination of *Luehea* (Tiliaceae) in a Costa Rican deciduous forest. *Ecology*, **63**, 1740–1750.

Jones FA, Hubbell SP (2003) Isolation and characterization of microsatellite loci in the tropical tree *Jacaranda copaia* (Bignoniaceae). *Molecular Ecology Notes*, **3**, 403–405.

Kandpal RP, Kandpal G, Weissman SM (1994) Construction of libraries enriched for sequence repeats and jumping clones, and hybridization selection for region-specific markers. *Proceedings of the National Academy of Sciences USA*, **91**, 88–92.

Kijas JMH, Fowler JCS, Garbett CA, Thomas MR (1994) Enrichment of microsatellites from the citrus genome using biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. *Biotechniques*, **16**, 657–660.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Zhang D, Hewitt GM (2003) Nuclear DNA analyses in genetic studies of populations: practice, problems, and prospects. *Molecular Ecology*, **12**, 563–584.