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TECHNICAL NOTE

Isolation and characterization of 15 polymorphic microsatellite loci in *Tetragastris panamensis* (Burseraceae), a widespread Neotropical forest tree

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Abstract *Tetagastris panamensis* is a tropical forest canopy tree that is broadly distributed across Central and South America. We isolated 15 microsatellite loci and characterized them in populations from Panama and Ecuador. The number of alleles per locus in 61 individuals ranged from 4 to 22, and expected heterozygosity ranged from 0.044 to 0.909. The high overall single-parent exclusion probability ($P = 1.00$) highlights the utility of these loci for parentage-based analyses of gene flow.

Keywords *Tetragastris* · Tropical forest · Barro Colorado Island · Yasuni · Forest dynamics plot

Tetragastris panamensis (Engler) O. Kuntze is a dioecious forest canopy tree of the Torchwood family (Burseraceae), whose geographic distribution ranges from Belize to French Guiana, Peru and Brazil (Croat 1978; Daly 1990). *T. panamensis* is morphologically and ecologically variable. It grows in a wide range of forest types from dry forest to pre-montane moist forest (sensu Holdridge et al. 1971) and exhibits variation in number and size of leaflets. In Panama, flowering of individual trees of *T. panamensis* may occur every 2 years from June to September, while fruits mature from March to May (Croat 1978). At maturity, the fruits dehisce to expose white arillate seeds that normally drop under parental crowns or are dispersed by monkeys and birds (Howe 1991, 1980). We developed microsatellite DNA markers for *T. panamensis* in order to investigate the contribution of vertebrates to gene flow, and to evaluate population structure and species boundaries at broader geographic scales.

We extracted DNA (DNeasy kit, Quiagen, Valencia, 41 California, USA) from two trees (plot ID # 259325 and 253390) from the mapped population of *T. panamensis* in the 50-ha Forest Dynamics Plot (FDP) in Barro Colorado Island (BCI), Panama for microsatellite development. Microsatellite markers were screened from a genomic library twice enriched for AG-, TG-, AAC-, AAG-, AAT-, ACT- and ATC-repeats, following the protocol of Glenn and Schable (2005). The enriched DNA was ligated into a cloning vector using the TOPO TA cloning kit (Invitrogen Corporation, Carlsbad, USA). Plasmid inserts were sequenced in both directions and screened for the presence of microsatellite repeats. Out of a total of 162 clones sequenced, 61 (38%) contained microsatellite repeats. Primers were designed for 34 of the 61 sequences, using the software Primer 3 v. 0.4.0. (Rozen and Skaletsky 2000) and OligoCalc (Kibbe 2007). Fifteen primer pairs produced robust PCR product and were used in subsequent genotyping.

To assess the polymorphism of the 15 loci we genotyped 61 individuals collected from Chiriqui, Panama ($N = 17$) (ca. 8°10′N, 82°25′W), Barro Colorado Island, Panama

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Table 1 Forward (F) and reverse (R) primer sequences, repeat motifs, number of alleles (N_a), allelic size range in bp (size range), observed heterozygosity (H_O), expected heterozygosity (H_E), and GenBank Accession number for 13 microsatellite loci for *Tetragastris panamensis* sampled from three geographic locations

Locus	Primer sequence (5′–3′)	Repeat motif	Size range (bp)	BCI ($n = 22$)			Chiriqui ($n = 17$)			Yasuni ($n = 22$)			Overall	Accession number
				N_a	H_O	H_E	N_a	H_O	H_E	N_a	H_O	H_E	N_a	
Tpan014	F: TAM—CAT CCA CCA AAT AAT GCG TTC R: CCA CCT CAA GGT AAC AAT G	(GT)15	139–171	6	0.636	0.608	5	0.647	0.701	8	0.364***	0.806	9	GQ470616
Tpan015	F: FAM—GGA GCC ACA TTA GTT CTC CTC R: GTA TCT CCC TCA GCT TCC AC	(AC)16	98–168	8	0.773	0.777	8	0.824*	0.732	15	0.909	0.909	22	GQ470612
Tpan031	F: TAM—TCA GAG CAC AAG TCC CGT AA R: TTC CTA GCC TTT GGT CTT GG	(AC)12	263–289	7	0.591	0.653	6	0.857	0.612	8	0.727**	0.673	10	GQ470613
Tpan152	F: TAM—CAC GGT CTC CCA CTT AGT CA R: GTG GAA ATT GTA TTC ATC CTT TTC T	(CA)13	265–305	9	0.409**	0.783	5	0.420	0.433	N/A	N/A	N/A	12	GQ470617
Tpan241	F: TGG CAT CTT GTT GAA ATA GTG G R: TGC ATA TGC TGG GTA GGA AG	(TG)15	282–320	7	0.909	0.779	6	0.933	0.762	7	0.500	0.775	13	GQ470614
Tpan301	F: FAM—ACG TGC ATG TGC CAT TAC AT R: TGA ACG ATA GAG CAG GAG GT	(AG)15	246–260	4	0.636	0.616	4	0.706	0.673	4	0.773	0.614	8	GQ470619
Tpan321	F: TAM—CCC TGG TGT TGT TGG ATT CT R: CAT CCC AAA ACT GAC GGA AT	(TC)11	271–295	2	0.045	0.044	4	0.647	0.555	5	0.500**	0.656	9	GQ470620
Tpan441	F: HEX—AAG CAC ATT CAG AAT TGA AGA A R: TAC TTG TGG TCCA CCA TCC T	(GA)10	260–290	7	0.500	0.573	7	0.824	0.782	8	0.727	0.750	13	GQ470622
Tpan681	F: TAM—GGA GAA GGT GAG AGT TGC AT R: GAG GTG AGC CTG AGA ACA AT	(AG)15(AG)15	264–286	5	0.409***	0.567	5	0.647	0.709	6	0.591***	0.637	8	GQ470623
Tpan882	F: GCG TGT GCT TAT CCA ACT CT R: TAM—CCT GCA AGT CAC ACG AAG AA	(TG)10(AG)8	256–264	2	0.364	0.496	2	0.118	0.208	3	0.182	0.168	4	GQ470624
Tpan890	F: GCA AAT GGG AAC CAG TTA AA R: HEX—CAC TTT TGG CCA GGT AAT TC	(AG)11	270–294	2	0.909***	0.496	3	0.176***	0.261	4	0.273***	0.594	5	GQ470615
Tpan893	F: CCC AAA ACC TAA AGC AGA TG R: HEX—CCA ACA ACA AAT CAA ACT CCT	(TTG)9	278–300	7	0.727**	0.777	7	0.882	0.820	3	0.318	0.429	12	GQ470625
Tpan896	F: FAM—TGC TCT CAA AAG TCC TGA TG R: GGG AAA GGG GAA GTA TCT TT	(AAG)10	240–278	4	0.684**	0.564	4	0.538	0.533	5	0.563	0.674	11	GQ475488

Significance level: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

($N = 22$) (9°9′N, 79°50′W), and Yasuní National Park, Ecuador ($N = 22$) (0°42′S, 76°23′W) (Table 1). PCR amplifications were performed in 10 µl reactions containing ca. 10 ng of genomic DNA, 1× PCR buffer, 0.2 nM dNTP, 1.25 mM MgCl2 and 1.25 U Taq polymerase (*GoTaq*, Promega, Madison, USA). The thermal cycle was set at 4 min at 94°C followed by 30 cycles of 30 s at 94°C, 40 s at 52°C (annealing temperature) and 60 s at 72°C, and a final extension step of 10 min at 72°C. Polymorphism of the loci was first assessed using dye labeled nucleotides (dNTPs). For polymorphic loci one of each primer pair was fluorescence-labeled with FAM, HEX or TAMRA (Table 1). PCR products were electrophoresed on an ABI 3730 Sequencer and analyzed using GeneMapper v. 4.0 (Applied Biosystems Incorporated, Foster City, USA).

Locus Tpan262 (F: TTT CTC AAC AGC GTC AGC AG, R: TTG TGG TAA ATA GCA TGG AAC G; repeat motif (AC)15; genbank accession GQ470618) was polymorphic but its chromatogram peaks were too complex to score and are presented in Table 1. Locus Tpan372 (F: TTT GTT TAG GTG GGT TCA AGG, R: TGT AAG TTG CAG TCA CGG AGA; repeat motif (CT)11; genbank accession GQ470621) was monomorphic in our sampled populations. Locus Tpan152 amplified in our two Panama populations, but did not amplify in DNA samples from Yasuní, which may reflect genomic divergence between the Panama and Ecuador populations. PCR amplification was successful in all populations for the 12 remaining primer pairs. The 13 loci polymorphic and scoreable loci had 4–22 alleles per locus in the overall sample (Table 1). The observed (H_O) and the expected (H_E) heterozygosity calculated using GenAlEx v. 6 (Peakall and Smouse 2006) ranged from 0.045 to 0.933 and 0.044 to 0.909, respectively. Hardy–Weinberg equilibrium (HWE) and linkage disequilibrum tests were carried out in FSTAT version 2.9.3.2, with significance levels adjusted using Bonferroni corrections for multiple tests. The 13 loci are unlinked. There were deviations from HWE expectations for two to four loci per population ($P < 0.05$). However, only three of the 13 loci (Tpan241, Tpan890 and Tpan014) showed significant deviations after correction for multiple tests, and only for the Yasuní population (Table 1). An estimation of the probability of null alleles using Micro-Checker v 2.2.3 (Oosterhout et al. 2004) indicated that null alleles may be present at these three loci as suggested by heterozygote deficiency (Table 1). The multilocus exclusion probability for one unkown parent was ca. 1.00 for each of the three populations, indicating high information content for parentage-based analyses of gene flow.

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