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Characterization of nuclear microsatellite loci in the Neotropical tree *Parkia panurensis* (Fabaceae)[1]

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- *Premise of the study:* We present here a set of nine polymorphic nuclear microsatellite loci, identified for the first time within the neotropical legume tree species *Parkia panurensis* Benth. ex H. C. Hopkins, which is widespread in western and central Amazonia.
- *Methods and Results:* To characterize these loci, 33 *Parkia panurensis* adult trees were analyzed. The number of alleles ranged from eight to 32, with an average of 14.4 alleles per locus. Mean expected heterozygosity ranged from 0.74 to 0.955.
- *Conclusions:* All nine loci could also be verified in six other *Parkia* species and polymorphic fragments amplified. The new marker set can be used for future studies of genetic diversity and differentiation, as well as estimation of gene flow and parentage analyses in various *Parkia* species.

Key words: cross-species amplification; *Parkia* species; seed dispersal; simple sequence repeats

Parkia is a tree species belonging to the genus of the legume family Fabaceae and has a pantropical distribution (Hopkins, 1986). One of the Amazonian species of *Parkia*, *P. panurensis* Benth. ex H. C. Hopkins, which is distributed over western and central Amazonia in Brazil, Peru, Colombia, and Venezuela (Hopkins, 1986), is a major food resource for the two tamarin species *Saguinus mystax* and *Saguinus fuscicollis* (Knogge and Heymann, 2003). In turn, these two primate species act as principal or—in the absence of woolly monkeys (*Lagothrix lagothricha*)—as exclusive seed dispersers for *P. panurensis* (unpublished data). The latter situation provides the opportunity to study the genetic consequences of seed dispersal in a simplified neotropical model, consisting of one plant and two disperser species. To analyze seed dispersal in *P. panurensis* in ongoing studies, nine nuclear microsatellite loci were identified in this species for the first time and are characterized here. Moreover, assignability to six other *Parkia* species was tested positively for all loci. These other species are *Parkia ingneiflora* Ducke, *Parkia multijuga* Bentham, *Parkia nitida* Miquel (all from the same location) and *Parkia plathycephala* Bentham, *Parkia bahiae* H.C. Hopkins and *Parkia pendula* (Willdenow) Bentham ex Walpers originating from different locations within South-America.

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METHODS AND RESULTS

An enriched library was constructed by ecogenics GmbH (Zurich, Switzerland) from size-selected genomic DNA ligated into SAULA/SAULB-linker (Armour et al., 1994) and enriched by magnetic bead selection with biotin-labeled $(GT)_{13}$, $(CT)_{13}$, $(GCT)_9$ and $(CGT)_8$ oligonucleotide repeats (Gautschi et al., 2000a; Gautschi et al., 2000b). Of 374 recombinant colonies screened, 166 gave a positive signal after hybridization. Plasmids from 72 positive clones were sequenced, and primers were designed for 20 microsatellite inserts. Amplification products of nine microsatellite markers showed scorable and polymorphic bands after running on an automatic capillary sequencer (MegaBACE 1000, GE Healthcare, Uppsala, Sweden). To characterize the nine confirmed loci, 33 *P. panurensis* adult trees from one single population at the Estación Biológica Quebrada Blanco in northeastern Peru (4°21′S, 73°09′W) were genotyped. DNA extraction followed the protocol by Dumolin et al. (1995). As a slight modification, the protocol included an additional final treatment with 0.5 μg RNase at 37°C for 30 min. PCR reactions were performed in a 25 μL volume containing 30 ng of template DNA, 1× PCR buffer (5× buffer Promega, Mannheim, Germany), 0.2 μM of each primer (forward primer fluorescence labeled), 0.2 mM of each dNTP, 2.0 mM $MgCl_2$, 1 unit/μl *GoTaq* polymerase (Promega, 5 units/μl) and 0.5% BSA (20 mg/ml). The thermal cycler (T1, Biometra, Goettingen, Germany) was programmed with the following conditions: 5 min at 95°C for denaturation, followed by 33 cycles at 95°C for 45 s, different annealing temperatures ranging from 54 to 60°C for 45 s depending on the specific primer pair used, elongation time at 72°C of 40 s and final extension at 72°C for 10 min. Annealing temperatures of the specific primer pairs for best amplification are summarized in Table 1. The PCR amplification products were separated by capillary electrophoresis using the MegaBACE 1000 automated sequencer (GE Healthcare). Alleles were sized using the size standard MegaBACE ET400-R (GE Healthcare) and the MegaBACE Genetic Profiler version 2. Primer labels are reported in Table 1.

Genetic diversity parameters for *P. panurensis* samples were estimated using GenAlEx version 6.2 (Peakall and Smouse, 2006). All nine loci were polymorphic with an average of 14.4 alleles per locus, ranging between eight and 32 (Table 2). Expected heterozygosity ranged from 0.74 to 0.955 with a mean value of 0.803. Significant deviations from expected heterozygote

Table 1. Characteristics of nine nuclear microsatellite loci developed in *Parkia panurensis*. Shown for each locus is the locus name, the forward (F, fluorescence labeled with HEX, FAM or TAMRA) and reverse (R) primer sequence, the optimized annealing temperature (T_a), the repeat motif, the size range of PCR products in base pairs and the GenBank accession number. Size ranges of the amplified fragments include all fragments discovered within all *Parkia* species used in this study. For explicit size ranges within a specific *Parkia* species see Table 2.

Locus	Primer sequence (5′ - 3′)	T_a (°C)	Repeat motif	Size range (bp)	GenBank ID
*Parpan*3	F: FAM-CACGTTAATTCAATCAAAATGGTG R: TTTTGCCTTTTTCGGACTTG	56.5	$(GT)_{15}$	155-209	GU735073
*Parpan*4	F: TAMRA-TTGATGGGAGTGGGAAAAAG R: CAGGAGGTGGTCTCTTCAGG	54.0	$(GT)_{13}(GA)_{15}$	148-210	GU735074
*Parpan*5	F: FAM-CTCAATAAGATACCCTTTACATTGC R: TTGAATCGAGGAATGAGATTATTG	60.0	$(CA)_{17}$	166-200	GU735075
*Parpan*9	F: FAM-GGGGCTTGTGTCTCTCACTG R: ACTTTGAAGGCACGAGATGG	58.0	$(AC)_{12}$	204-262	GU735076
*Parpan*11	F: HEX-ACGTAGGGAATAGGGCCATC R: CTACGTACGAGCCGACACTC	58.5	$(TG)_5CA(TG)_{16}$	94-214	GU735077
*Parpan*13	F: TAMRA-CCTCCCTCGCTTCACAATC R: CACATGCAAATGAAAATGGTG	58.5	$(GT)_{17}TT(GT)_8$	86-194	GU735078
*Parpan*14	F: HEX-ACATCAAAATGGTCGCTCAAC R: CAAATGTTCTTGTATGGAGCAAG	60.0	$(GT)_{20}$	76-116	GU735079
*Parpan*15	F: HEX-TGGCCTCACTGCATACTGAC R: TGGGATGAACAAAACTGTGC	55.0	$(AC)_{24}$	104-152	GU735080
*Parpan*21	F: HEX-TGCTTTGTGCGACTTGAATC R: CATTGTTCAGCATATAGGCATACAG	58.0	$(GT)_{15}$	153-185	GU735081

Table 2. Results of primer application in seven different *Parkia* species. The primers were originally developed in *Parkia panurensis.* For each locus the following informations are shown locus name, number of alleles (N_a), size range of PCR products in base pairs and expected heterozygosity (H_e). The sample size for each species is given in parentheses behind the species name. The second line shows the geographic coordinates of each study site, where the samples came from.

Results for samples originating from a mixed stand of four different *Parkia* species												
	P. panurensis (33) 4°21’45″S 73°09’30″W			*P. ingneiflora* (26) 4°21’45″S 73°09’30″W			*P. multijuga* (21) 4°21’45″S 73°09’30″W			*P. nitida* (19) 4°21’45″S 73°09’30″W		
Locus	N_a	bp	H_e	N_a	Bp	H_e	N_a	bp	H_e	N_a	bp	H_e
*Parpan*3	14	155-193	0.838*	17	155-209	0.862**	14	155-201	0.798 ns	10	155-183	0.662***
*Parpan*4	17	158-196	0.860 ns	20	148-210	0.928 ns	10	142-172	0.747*	8	144-178	0.735*
*Parpan*5	13	168-198	0.792*	9	166-188	0.751***	8	168-200	0.791***	10	168-194	0.832*
*Parpan*9	13	204-256	0.823 ns	12	216-262	0.758*	10	210-248	0.834**	8	232-254	0.759 ns
*Parpan*11	10	142-192	0.760***	6	148-192	0.650***	13	94-208	0.717***	6	142-214	0.715***
*Parpan*13	32	98-194	0.955***	9	96-174	0.548***	12	102-132	0.873*	10	86-124	0.846*
*Parpan*14	9	86-116	0.584**	11	78-108	0.839***	5	76-94	0.584***	11	82-110	0.835***
*Parpan*15	14	128-160	0.872 ns	13	128-152	0.838 ns	13	104-152	0.891*	6	128-144	0.673 ns
*Parpan*21	8	157-173	0.740 ns	4	157-181	0.510 ns	5	157-185	0.645 ns	14	155-213	0.886**
Results for three additional *Parkia* species originating from different sites												
	P. plathycephala (9) 07°19’04″S; 39°24’07″W			*P. bahiae* (4) 13°41′52″S 39°00′58″W			*P. pendula* (12) 13°46′11″S 39°10′44″W					
Locus	N_a	bp	H_e	N_a	bp	H_e	N_a	bp	H_e			
*Parpan*3	10	171-207	0.870 ns	2	179-183	0.219 ns	6	165-187	0.722*			
*Parpan*4	4	176-194	0.543 ns	3	162-168	0.594 ns	4	180-188	0.462**			
*Parpan*5	4	168-184	0.539***	4	168-174	0.688 ns	7	170-186	0.792 ns			
*Parpan*9	1	232[a]	—[b]	3	240-246	0.594 ns	6	224-256	0.733*			
*Parpan*11	4	148-192	0.512*	2	148-192	0.375 ns	6	146-192	0.711*			
*Parpan*13	5	96-108	0.698 ns	2	118-122	0.500*	7	102-130	0.663***			
*Parpan*14	5	88-112	0.667 ns	2	84-90	0.219 ns	5	84-112	0.649**			
*Parpan*15	3	104-140	0.426*	3	136-140	0.625*	3	136-144	0.486**			
*Parpan*21	3	159-163	0.494 ns	3	163-171	0.406 ns	4	153-161	0.587*			

Notes: *, ** and ***: significant departures from Hardy-Weinberg equilibrium at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.
n.s. = not significant.
[a]all individuals are homozygous.
[b]H_e cannot be calculated because the marker is monomorph in this species.

frequencies at Hardy-Weinberg equilibrium were observed for Parpan3 and Parpan5 ($P < 0.05$), Parpan14 ($P < 0.01$) and Parpan11 and 13 ($P < 0.001$).

In an additional step, the newly developed nine microsatellite markers were tested in six other neotropical *Parkia* species with individual numbers ranging from 4 to 26 (Table 2). Cross-species amplification was found for all nine loci in all tested species. In either case loci were polymorphic and showed amplified DNA fragments of similar sizes compared to *P. panurensis* (Table 2). However, one exception occurred at locus Parpan9 in species *P. plathycephala,* where only one allele

was found. This might be an effect of the sample size and might disappear in real population genetic studies.

CONCLUSIONS

In future analyses, the developed microsatellite markers will be used to study small-scale genetic structure as well as seed- and pollen-mediated gene flow of various *Parkia* species in northeastern Peru.

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