American Journal of Botany

American Journal of Botany: e105–e106. 2010.

AJB Primer Notes & Protocols in the Plant Sciences

Characterization of 14 microsatellite loci in a tropical palm, *Attalea phalerata* (Arecaceae)[1]

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- *Premise of the study:* We developed microsatellite primers for the widely distributed tropical palm *Attalea phalerata* for studies on the dispersal and spatial genetic structure of palm populations.
- *Methods and Results:* Fourteen di-, tri-, and tetra-nucleotide microsatellite primer pairs were identified. The number of alleles in the population tested ranged between 3 and 25, with a mean of 12.1. Ten microsatellite loci exhibited no significant deviations from Hardy–Weinberg Equilibrium or presence of null alleles, and their combined probability of exclusion was 0.998.
- *Conclusions:* These microsatellite loci will be useful in parentage analysis and population genetics studies of *Attalea phalerata*.

Key words: Arecaceae; *Attalea phalerata*; microsatellite.

Attalea phalerata Mart. ex Spreng. is widely distributed along the periphery of the southern and western Amazonia (Henderson et al., 1995). It is a monoecious but functionally dioecious palm; male and female flowers on each palm flowers asynchronously, which prevents self-fertilization (Pintaud, 2008). *Attalea phalerata* is also ecologically and culturally important. A diversity of frugivores, including scatter-hoarding rodents (e.g., *Agouti paca*, *Dasyprocta punctata*, *Sciurus* spp.), primates (e.g., *Cebus apella*), and tapirs (*Tapirus terrestris*) depend on the fruit resources of *A. phalerata*, and they also disperse the seeds of this palm (Quiroga-Castro and Roldán, 2001; Choo, 2010). Although palms in the genus *Attalea* produce seeds with stony endocarps, undispersed seeds or those found near maternal trees suffer high rates of mortality from the predatory activities of bruchid beetles (Wilson and Janzen, 1972; Wright et al., 2000). The loss of frugivore dispersers through anthropogenic activities, especially hunting, will likely have significant impacts on the dispersal, recruitment success, and population genetics for *A. phalerata*. Here, we characterized 14 polymorphic loci for *A. phalerata* that will be useful for determining the parentage of dispersed seeds and seedlings and understanding the genetic impacts of hunting on the dispersal and population genetic structure of palm populations.

METHODS AND RESULTS

To construct the genomic library, leaf tissues were obtained from a single *Attalea phalerata* palm individual (voucher deposited at TEX under *J. Choo 2007-12-5001*) collected within a 2.25-hectare study plot established at the Cocha Cashu Field Station, Madre de Dios, Peru (11°54'S, 71°22'W). Palms found within this study plot represent natural stands. DNA from the leaf tissues was extracted using the DNeasy plant tissue kit (QIAGEN, Valencia, CA). The microsatellite libraries were developed by Genetic Identification Services (Chatsworth, CA) following the methods of Jones et al. (2002) and from approximately 100 ng of genomic DNA. The libraries were enriched for four repeat motifs—$(GA)_n$, $(CA)_n$, $(AAC)_n$, and $(TAGA)_n$. One hundred clones were sequenced, and primer pairs were designed for 75 unique sequences using DesignerPCR version 1.03 (Research Genetics, Huntsville, AL). We added a HEX-labeled M13 tail to each forward primer (5′-TGT AAA ACG ACG GCC AGT-3′) following Schuelke (2000).

We used 36 individual *Attalea phalerata* palms, which included 32 reproductive adults, to test the amplification and polymorphism of each locus. DNA from these individuals was extracted from silica-dried leaf tissues using a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). PCR was conducted for individual pairs of primers in 10-µl reaction volumes containing 20 ng/ul of template DNA, 1× PCR buffer, 2.5 mM MgCl, 1 mg/ml BSA, 0.03 µM forward primer, 0.25 µM reverse primer, 0.4 µM forward dye-labeled M13 primer, 0.2 mM dNTPs and 1U Taq polymerase. The PCR conditions were 94°C for 2 min, 30 cycles at 94°C for 45 s, 55°C for 45 s, 72°C for 30 s, and a final extension of 72°C for 10 min. We ran PCR products on a 2% agarose gel stained with SYBR safe (Invitrogen, Carlsbad, CA) and used UV light to visualize them.

Fourteen loci showed reliable amplification and allelelic polymorphisms that we could score consistently (Table 1). For these 14 loci, we replaced the general M13 forward with individually labeled fluorescent primers and grouped them into three multiplex PCR reactions, namely P, Q, and R. Multiplex P was comprised of primer B121_4, C11_7, D106_3, and D124_5; multiplex Q included A106_2, C5_4, D2_1, D3_2, and D110_8; and multiplex R was composed of A103_3, B101_4, B102_8, B103_3, and C122_3. The multiplex PCR conditions were: 94°C for 3 min, 30 cycles each of 94°C for 30 s, 52°C (multiplex P)/63°C (multiplex Q)/60°C (multiplex R) for 45 s, and 72°C for 45 s, and a final extension of 72°C for 10 min. PCR products were analyzed on an ABI 3100 Genetic Analyzer, and alleles were scored using SoftGenetics GeneMarker v1.7 (State College, PA). For each locus, we tested for presence of null alleles and deviations from Hardy–Weinberg Equilibrium (CERVUS; Kalinowski et al., 2007).

We tested the multiplexed primers on 569 *Attalea phalerata* individuals in a population of palms in a 2.25-hectare experimental plot at Cocha Cashu. All 14 loci were polymorphic, with the numbers of alleles per locus ranging between 3 and 25; the average number of alleles was 12.1 (Table 1). The observed heterozygosity ranged between 0.26 and 0.89, with a mean of 0.68. Four loci,

[1] Manuscript received 28 July 2010; revision accepted 23 August 2010.

JC thanks P. Jordano and J. Godoy for technical advice and S. Boles for her support in the laboratory. JC also acknowledges financial support from the University of Texas at Austin and the Lindbergh Foundation.

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doi:10.3732/ajb.1000281

TABLE 1. Characteristics of 14 polymorphic microsatellite loci amplified in *Attalea phalerata.*

Locus (GenBankID)	Repeat motif	Primer sequences (5′–3′)	Size (bp)	T_a	Ho	He	HWE	No. of Alleles
A103_3 (HM563062)	GT_9	F: CAATGCAAGAGACAAGCATAC R: GCACACTTGATGACATTTTATG	235-259	59.9	0.758	0.767	NS	8
A106_2 (HM563063)	CA_{11}	F: CATTTGGCATTCTTACACATTC R: CTTGGGGTGAACTGACTTTAC	158-178	60.8	0.751	0.743	NS	8
B101_4 (HM563064)	TC_{19}	F: CCTGGTCATCCGATTATTTCA R: TGTCGCCATTCTTTCGTTTAT	132-166	63.4	0.797	0.814	NS	17
B102_8 (HM563065)	GA_{21}	F: AGCACTAATGTGCATGTATGTG R: CCATTCCCTCTACAAGGATAAC	160-190	60.1	0.841	0.843	NS	17
B103_3 (HM563066)	TC_{17}	F: ATGCTGCTTGCGGTGTAG R: GAGGTATTGATGGGAGGAAGAC	192-232	62.7	0.836	0.871	NS	16
B121_4 (HM563067)	TC_{12}	F: CCTGGAGCATCAATGGAC R: TCCGAGAACCCTAAACCTG	121-147	61.7	0.869	0.845	NS	12
C5_4 (HM563068)	GTT_9	F: AAGATGACCGTAGCATTAACAG R: TCCCATGTTTTCTTTAGTCTTC	251-275	59.7	0.646	0.681	NS	9
C11_7 (HM563069)	CAA_9	F: AGTCGTGAACTCTACCACTTTC R: TGTTGCCCTTCAGATATAGATC	214-238	58.9	0.633	0.649	NS	9
C122_3‡ (HM563070)	CAA_8	F: TCCTCCACCTCCAATGGTAG R: TTCGACATGACGAGAACGTC	257-275	63.8	0.541	0.65	*†	8
D2_1‡ (HM563071)	$ATCT_7$	F: CTGTCCTGCACAAGAAGTTGA R: AAGTTGGCGCATATCAATGAC	200-217	63	0.688	0.463	*†	3
D3_2‡ (HM563072)	$ATCT_5$	F: ACAGGTGTGGTTCAATCAAT R: GGAGGGATAGACAAAGGAAG	228-283	59.3	0.26	0.61	*†	10
D106_3 (HM563073)	$AGAT_7$	F: ACCACCCCATCACAAAAG R: GGACCATTTCAGCCAGAG	171-215	60.8	0.557	0.611	NS	10
D110_8‡ (HM563074)	$ATCT_7$	F: GCAGTGGTATGCCTGTATAGTG R: CTAGCACACATGCACACATG	163-201	61	0.435	0.74	*†	17
D124_5 (HM563075)	$ATCT_{10}$	F: GGTGGTGATTGAACTGAACTC R: GCTGATGCTTGCTGACAG	233-299	61	0.891	0.881	NS	25

T_a = annealing temperature; H_o = observed heterozygosity, H_e= expected heterozygosity, HWE = Hardy–Weinberg Equilibrium test, NS = not significant

*Significant $P < 0.0000001$; †Significant presence of null alleles; ‡Primer pairs that exhibited significant deviations from HWE and significant presence of null alleles.

D2, D3, D110, and C122, showed significant departures from Hardy–Weinberg equilibrium and null-allele frequencies greater than 0.05 (Table 1). These four loci should therefore only be included for parentage analysis with programs that can accommodate bias estimates associated with null alleles (Pemberton et al., 1995; van Oosterhout et al., 2004). The remaining 10 microsatellite loci have a combined probability of exclusion of 0.998 for the first parent and thus provide considerable power for parentage studies (CERVUS; Kalinowski et al., 2007).

CONCLUSIONS

Of the 14 microsatellite markers developed for *Attalea phalerata* parentage, 10 were found to be reliable and sufficiently polymorphic for parentage analysis of *Attalea phalerata* individuals within a population. These markers will be useful for local-scale studies aimed at inferring the relative contribution of seed dispersal and/or pollination to gene flow and population genetic diversity.

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