30 s followed by a final extension at 72 °C for 10 min. Amplified fragments were analysed on an ABI PRISM 377 Genetic Analyser, with ROX 350 (Applied Biosystems) as the internal size standard. GeneScan 3.7 and GenoTyper 3.7 (both are Applied Biosystems) were used to output score allelic data. Observed and expected heterozygosities for each locus were calculated by Cervus 3.0 (Kalinowski *et al*. 2007). GenePop 3.4 (Raymond & Rousset 1995) was used to test for deviation from Hardy–Weinberg equilibrium and linkage disequilibrium. Characteristics of 10 polymorphic microsatellite loci from the Chinese alligator are described in Table 1.

The number of alleles per locus ranged from one to seven in these two populations, whereas the ranges of expected and observed heterozygosities are from 0 to 0.784 and from 0 to 0.500 in Xuanzhou captive population, while these two data are from 0.089 to 0.692 and from 0 to 0.636 in the wild population, respectively. Of the 10 loci, no significant pairwise linkage disequilibrium ($P < 0.01$) was detected across both populations. Since two loci (Asiu4 and Asiu234) of the Xuanzhou captive population were monomorphic loci, Hardy–Weinberg equilibrium cannot be calculated. Four loci (Asiu2, Asiu44, Asiu96, Asiu122) in the captive population and nearly all the loci in the wild population except two loci (Asiu1 and Asiu122) deviated significantly from Hardy–Weinberg equilibrium ($P < 0.01$). This might be caused by null alleles, nonrandom sampling, mating systems or inbreeding. Our studies suggest that these microsatellite loci are suitable for population genetic analysis and paternity study on *A. sinensis* in the future.

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Characterization of polymorphic microsatellite loci in *Yucca filamentosa*

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Abstract

Yuccas and their pollinator moths are a textbook example of mutualism, yet we lack sufficiently variable markers to properly study the population genetics of the plants. We characterized 13 polymorphic microsatellite loci for *Yucca filamentosa* by screening primers derived from an expressed sequence tag database. We found four to 13 alleles per locus and the observed heterozygosity ranged from 0.31 to 1. These markers will be useful in future ecological studies of *Y. filamentosa*.

Keywords: Agavaceae, microsatellite, mutualism, PCR primers, *Yucca*

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Table 1 Primer information and characteristics of 13 polymorphic microsatellite loci in *Yucca filamentosa*

Unigene no.	Primer name	Primer sequence (5′–3′)	Repeat motif	T_a (°C)	No. ind.	Size range (bp)	N_a	H_O/ H_E	PIC	HWE	GenBank Accession no.
274075	Msat-3	F: AGGCAGCTAGGTCAGGATCA	$(TGA)_6$	60	28	234–257	5	0.86	0.57	0.1321	EU867774
		R: GCTCAACTGAAACCAGCACA						0.63			
275339	Msat-6	F: CTGGTTCCCTCCAACCTACA	$(AG)_{18}$	59	25	146–182	13	0.96	0.88	0.7411	EU867775
		R: CCCTTCAGTTTGTTGATGCAC						0.91			
274087	Msat-11	F: AACGCAATCCACAACAAACA	$(GAG)_7$	59	34	149–195	12	0.94	0.86	0.0276	EU867776
		R: GGGGTTTTGATCAGCTTCAA						0.89			
274545	Msat-14	F: ATTCCCATCCATCATCCAAA	$(CAT)_8$	56	32	192–216	7	0.75	0.60	0.6973	EU867777
		R: TACCTCTTGCTGCTGCTCCT						0.67			
274087	Msat-18	F: GAGAGGAGGAGGAGGAGGAG	$(AGC)_9$	62	34	153–208	10	1.00	0.80	0.0193	EU867778
		R: TGGGCATCATCATTGCTCTA						0.84			
274393	Msat-20	F: TTGCATGAAGCATGATGTTG	$(TG)_8$	56	31	115–139	11	0.90	0.69	0.7273	EU867779
		R: GCATCCACACATACGCACTC						0.72			
274506	Msat-21	F: CCTCCCTTTTGCTCTCCTCT	$(TC)_{13}$	61	32	174–216	11	0.88	0.85	0.0053	EU867780
		R: TCAGCAAGATGTGGTTGCTC						0.88			
274517	Msat-22	F: CACCCTTACACTCCCACCAG	$(CAA)_7$	57	29	208–219	4	0.31	0.34	0.3173	EU867781
		R: TGCCTGACTAGGTCCTCAAAA						0.36			
274555	Msat-23	F: CGGCGAGATTTCTACTGAGG	$(CTG)_5$	62	27	200–214	4	0.41	0.33	1.0000	EU867782
		R: AGTTTGCCACTTGGGTGTCT						0.36			
275275	Msat-25	F: CATGGAAAGAAAATGGAGAAAGA	$(AAC)_5$	60	30	230–248	5	0.70	0.51	0.6210	EU867783
		R: TGGATGGAGGTGATACGACA						0.57			
275022	Msat-28	F: GGCAAAACTCTAAATACTCCATTTC	$(CT)_{10}$	58	32	160–174	11	1.00	0.85	0.3815	EU867784
		R: ATCGGGAGATGGAATTAGGG						0.88			
274063	Msat-32	F: GGTTCAAAGGGGCTGTTTCT	$(CT)_4$	56	29	120–186	4	0.55	0.37	0.4527	EU867785
		R: TTAATGGCCGGAATTCAAAG						0.43			
274027	Msat-36	F: TACCCGTTCTTGCGGATAGT	$(CT)_9$	60	34	136–162	10	0.94	0.74	0.0928	EU867786
		R: GCTGAGTTCATCGTCGTCCT						0.78			

T_a, annealing temperature; No. ind., number of individuals genotyped in the sample; N_a, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphic information content; HWE, *P* value of the Hardy–Weinberg test.

Yuccas are well known for their pollination mutualism with yucca moths and this system has been used frequently as a model in theoretical and empirical studies of the ecology and evolution of mutualisms (Pellmyr 2003). Since the original description in the 1800s by Riley (1872), a majority of the ecological work on this interaction has been conducted on only a handful of yucca species. One of these, *Yucca filamentosa*, was the subject of Riley's observations and has been integral in furthering research on the costs/benefits and regulatory mechanisms of the mutualism, the evolution of cheater species, and incipient speciation (e.g. Pellmyr & Huth 1994; Leebens-Mack & Pellmyr 2004; Segraves & Pellmyr 2004). Although phylogeographical and population genetic studies abound for the pollinators and the other specialized prodoxid moths that also feed on yuccas (e.g. Althoff *et al*. 2001; Leebens-Mack & Pellmyr 2004; Segraves & Pellmyr 2004), the lack of sufficiently variable genetic markers has hindered similar progress for the plants. Here we identify 13 polymorphic microsatellite markers suitable for population genetic studies and paternity analysis.

We used a previously developed expressed sequence tag (EST) database available on the Plant Genome Network (http://pgn.cornell.edu) to make an initial screen for candidate markers. A total of 1743 ESTs were sequenced from a cDNA library derived from *Y. filamentosa* flower buds. ESTs were assembled into 1490 unigenes including 153 multisequence contigs and 1337 singletons. Methods for cDNA library construction and EST processing followed Albert *et al*. (2005). Unigenes were screened for simple repeats using the Simple Sequence Repeat Identification Tool Perl script (Temnykh *et al*. 2001). Primers were designed using Primer3 (Rozen & Skaletsky 2000).

We screened 36 candidate loci for polymorphism in 25–34 naturally occurring plants collected from the Archbold Biological Station in Lake Placid, Florida, USA. Leaf tissue was kept at –80 °C until extraction with the Promega Wizard Genomic DNA purification kit. Polymerase chain reaction (PCR) was conducted in 30 μL reaction volumes containing 1× Fisher PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.3 μM each primer, 1 U *Taq* polymerase, and approximately 10 ng DNA for 35 cycles at 95 °C for 60 s,

56–62 °C for 60 s, and 72 °C for 90 s (see Table 1 for exact annealing temperatures). Products were electrophoresed on 5% Amresco SFR agarose and on 10% Biorad acrylamide gels to initially screen for polymorphism in eight to 14 individuals. If polymorphism was indicated, PCR was repeated with fluorescently labelled forward primers and the products were separated on an ABI 3730xl fragment analyser. Alleles were scored using GeneMapper version 3.0 and were checked visually. We verified the presence of microsatellites by direct sequencing. Unlabelled PCR products were cleaned using QIAGEN PCR purification columns. Dye terminator reactions were carried out following the Big Dye protocol (Applied Biosystems) in one-sixteenth recommended reaction volumes. Dye terminator reactions were cycled 24 times at 96 °C for 15 s, 50 °C for 15 s, and 60 °C for 4 min. The resulting products were sent to the Cornell Life Sciences Core Laboratory for sequencing. Sequences were edited in Sequencher version 4.5.

From the initial 36 loci, 24 consistently amplified a single product. Of these loci, 15 exhibited potential polymorphism in initial screens, and secondary screens indicated that 13 were polymorphic. We determined the observed (H_O) and expected (H_E) heterozygosities and polymorphic information content (Table 1) using Cervus version 2.0 (Kalinowski *et al*. 2007). The frequency of null alleles was zero for all loci except one that had an estimated frequency of 0.08. Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were assessed using GenePop version 4.0 (Rousset 2007). The resulting P values were Bonferroni-corrected for multiple comparisons (HWE: $P < 0.004$; LD: $P < 0.0006$). After correction, zero loci showed significant departures from HWE (Table 1), and significant LD was indicated for only one pair of loci: Msat-18 and Msat-36. Prior to correcting for multiple tests, significant LD was indicated in 21 of 78 pairs. Of these 21 pairs, two loci are linked as the primers were located in the same unigene build (Msat-1 and Msat-18; $P = 0.009$). A screen of 225 *Yucca brevifolia* showed significant polymorphism for at least five of these loci (J. Yoder, personal communication; Msat-6, 22 alleles, $H_O = 0.72$; Msat-11, eight alleles, $H_O = 0.65$; Msat-18, 12 alleles, $H_O = 0.49$; Msat-21, 13 alleles, $H_O = 0.51$; Msat-36, 17 alleles, $H_O = 0.56$). *Y. brevifolia* and *Y. filamentosa* are distantly related yuccas, suggesting that some of these markers may be useful across *Yucca* and perhaps for other Agavaceae. Furthermore, we anticipate that these markers will be extremely useful in furthering our understanding of the plant side of this model mutualism.

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