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PRIMER NOTE

Characterization of microsatellite loci in Kearney's bluestar (*Amsonia kearneyana*) and cross-amplification in other *Amsonia* species

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Abstract

Kearney's bluestar (*Amsonia kearneyana*) is a highly endangered herbaceous perennial in the family Apocynaceae. The species is found only in the Baboquivari Mountains of southern Arizona. We report the isolation and development of 12 microsatellite loci for Kearney's bluestar. Numbers of alleles ranged from two to four and observed heterozygosities ranged from 0.20 to 0.80 in the nine loci found to be polymorphic in the test population. All loci were also tested for cross-amplification in five other *Amsonia* species representing two subgenera from the southwestern United States. Some loci that were not polymorphic in the Kearney's bluestar were polymorphic in other species.

Keywords: *Amsonia kearneyana*, Apocynaceae, Kearney's bluestar, microsatellite

Kearney's bluestar is a federally endangered plant endemic to the Baboquivari mountains of Arizona. There are fewer than 1000 known plants, distributed among three drainages of Baboquivari Peak. Population sizes range from less than 10 plants to several hundred plants. Individual plants are long lived and produce many ramets from a single large fibrous root. The lifespan of mature *Amsonia* spp. plants is not well established, but may be many decades in length. Consistently small and highly dispersed populations and a paucity of discrete differentiating characters have contributed to significant taxonomic revisions in the genus (Woodson 1928; McLaughlin 1982). Most of the known populations of Kearney's bluestar are very small and contain fewer individuals than are necessary for the long-term maintenance of genetic diversity. We have developed these microsatellite markers to evaluate the levels and distribution of genetic variability among populations of Kearney's bluestar as part of an ongoing project investigating the ecology of the species.

We combined DNA from two different species prior to beginning the library construction process to maximize cost and time efficiency. As the frequency of repeat motifs varies in different taxa, we used enrichment procedures for

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four different repeat motifs in order to create four composite libraries that were preferentially enriched for one of the two species. We purified genomic DNA from silica-gel-dried leaf samples of *A. kearneyana* using the CTAB method (Doyle & Doyle 1987), followed by phenol-chloroform extraction and ethanol precipitation, and from the blood of the northern goshawk (*Accipiter gentilis*) using Qiagen Dneasy® Kits, and combined the DNA in equal proportions. Libraries enriched for $(GA)_n$ $(TAGA)_n$ $(TACA)_n$ and $(AAT)_n$ repeat motifs were constructed by Genetic Identification Services (Chatham, CA) and screened according to the protocol in Meredith & May (2002). Five hundred and seventy-two plasmids were purified using Qiagen Qiaprep® Kit and cycle sequenced using ABI BigDye™ Terminator Cycle Sequencing Ready Reaction kit version 1.0 and pUC19 forward and / or reverse sequencing primers. This total was comprised of 211 clones from the $(GA)_n$ library, 187 from the $(AAT)_n$ library, and 87 from each of the $(TAGA)_n$ and $(TACA)_n$ libraries. Sequencing reactions were purified using RapXtract™ magnetic bead separation (Prolinx) and separated on the MJ Research Basestation™ as described in Welsh *et al.* (2003). We assembled sequences using SEQMAN™, and identified 46 different microsatellite repeats and designed PCR primer pairs for each using PRIMERSELECT™ (Lasergene 5.1, DNASTAR Inc.).

Primer pairs were designed to amplify fragments from 150 to 250 basepairs in length.

As the libraries were composites of two distinct species DNA, we initially screened primer pairs against four northern goshawk samples and four samples of Kearney's bluestar. Five ng of genomic DNA were used as a template in a 10 μL reaction with 10 mM Tris-HCl, pH 9.0; 50 mM KCl; 0.1% Triton® X-100; 1.5 mM MgCl$_2$; 0.2 mM each dNTP; 1 mM unlabelled forward and reverse primers; and 0.375 units *Taq* polymerase (Promega™). Amplification was conducted using a touchdown protocol with the following parameters: 95 °C for 1 minute followed by 30 cycles of 95 °C for 1 minute, 67 °C for 45 s with a 0.5 °C decrease each cycle, and 72 °C for 2 min. PCR product was diluted 1:1 with 98% formamide loading buffer (10 mM EDTA pH 8.0; 0.005% xylene cyanol and 0.005% bromophenol blue) denatured at 95 °C for 3 min and chilled on ice prior to loading. Samples were separated on a 5% polyacrylamide gel at 50 W for 70 min. Gels were stained using the Sybr-Green™-agarose overlay protocol described in Rodzen *et al*. (1998) and scanned on a Molecular Dynamics™ FluorImager 595.

Of the 46 primer pairs designed, 19 amplified appropriately sized fragments in Kearney's bluestar and 18

amplified fragments in the northern goshawk (J.R. Topinka, manuscript in preparation). The remainder either did not amplify or amplified many nonspecific bands and did not result in robust and specific amplification upon further optimization. Candidate primer pairs were screened for polymorphism against 10 individuals selected from the largest known Kearney's bluestar population ($n \cong 300$), as well as two individuals from each of two different populations chosen to represent the complete geographical range. Expected and observed heterozygosities, and allele numbers are reported from the 10 individuals from the largest population (Table 1). In the test population, allele numbers for loci ranged from one to four alleles per locus (mean = 2.45) and expected heterozygosities ranged from 0.00 to 0.69 (mean = 0.46). Tests for Hardy–Weinberg equilibrium (HWE) were conducted in GENEPOP 3.3 using exact tests (Raymond & Rousset 1995). Locus Ake-4 was significantly out of HWE ($P < 0.05$). This departure from HWE may be due to the presence of a null allele at this locus and will require further analysis. After Bonferroni correction, no significant linkage disequilibrium was detected using Fisher's exact test conducted in GENEPOP 3.3 using the default Markov chain parameters. Because we are also

Table 1 Primer sequences for *Amsonia kearneyana* microsatellite loci

Locus	Repeat motif	Primer sequence (5′–3′)	Product size (bp)	GenBank Accession #	n	# of alleles	H_O	H_E
Ake1	(CT)$_{19}$	F-CGACTCACTTCTCTGTTCATTCTC	170	AY312439	10	3	0.50	0.64
		R-ACTACTTGGTGGTTGCTGGTG						
Ake2	(CT)$_{19}$(CTCTT)$_5$	F-ATATCAGTTTGAAAGTCCAGTGTC	155	AY312440	10	2	0.70	0.48
		R-ATTCATCGGCAATTCAGTAGA						
Ake3	(CT)$_3$ … (CT)$_{10}$	F-TCGACGCTGAGATTGACTGCTTAG	200	AY312441	10	3	0.60	0.69
		R- GCCTGACTTCACATTACAAACAAA						
Ake4	(CT)$_{18}$	F-CAGTTTCTTTGGAGTGGTG	179	AY312442	6	2	0.00#	0.48
		R-CAATCAGATAGGCAACGAG						
Ake5*	(CT)$_{16}$ … (CA)$_6$(TA)$_2$	F-CGTAGACGATAGCCTTGATAGC	232	AY312443	10	1	0.00	0.00
		R-CACTCCTGGGATGCTTTCA						
Ake6	(CT)$_{13}$ … (CT)$_2$(CA)$_9$	F-CAGAGACAATGATAAGACCACAAT	169	AY312444	10	3	0.80	0.64
		R-ATACCATGAAAAGGCTGCTC						
Ake7	(CCT)$_5$(CAT)$_{10}$	F-CCAAAACGCCCAAGTAAAATG	212	AY312445	10	3	0.80	0.59
		R-CGACGAAACGAGCGAAGAA						
Ake8	(GA)$_{23}$	F-ATGGCTGCAAAAGATGAAG	125	AY312446	10	4	0.60	0.62
		R-AGAGGGAGATAAACCGTAAGAATA						
Ake9	(CT)$_{19}$	F-GAAGCAATCAAGCAAGAAAT	226	AY312447	10	3	0.50	0.42
		R-ATAACCAAACAGGCTAAATA						
Ake10	(AAT)$_9$	F-AAAAACGAATCGAAAAACATAATA	231	AY312448	10	2	0.20	0.54
		R-GCGATCATACCAGCACTAAT						
Ake11*†	(GA)$_{13}$	F-TCCTTTGTAAAGCCGTGAGATT	122	AY312449	10	1	0.00	0.00
		R-TTTCCCCTTTTTGCTTGATTT						
Ake12*†	(GA)$_{30}$	F-GTGTTTGGGTTTTAAGGAAGAA	226	AY312450	2	1	0.00	0.00
		R-AAAGCCCAACTAAATATAACTAAT						

n, number of individuals analysed; H_O, observed heterozygosity, H_E, expected heterozygosity; F, forward primer; R, reverse primer; interrupted microsatellites are indicates by a (…) between motifs; *polymorphic in other *Amsonia* spp.; †polymorphic in other *A. kearneyana* populations; # significant departure from Hardy–Weinberg equilibrium ($P < 0.05$).

Table 2 Cross-species amplification of Amsonia kearneyana microsatellite primers

	Species				
Locus	*A. palmeri*	*A. peeblesii*	*A. tharpii*	*A. jonesii*	*A. grandiflora*
Ake1	2	2	2	2	2
Ake2	–	–	–	–	–
Ake3	3	1	1	3	2
Ake4	3	3	1	1	3
Ake5	2	3	2	4	3
Ake6	+ –	+ –	+ –	+ –	+ –
Ake7	4	2	3	2	1
Ake8	5	3	2	+ –	1
Ake9	2	2	2	–	1
Ake10	ns	ns	ns	ns	ns
Ake11	2	+ –	–	+ –	–
Ake12	+ –	2	2	+ –	1

To test for cross-species amplification, all 12 primers pairs were screened against DNA from three individuals of each species. Numbers indicate strong amplification and the total number of alleles that amplified; ns, nonspecific amplification; ±, weak amplification; –, no amplification.

interested in using these microsatellites to examine the distribution of genetic diversity in other species of *Amsonia*, we screened all primer pairs that successfully amplified against a suite of *Amsonia* species, regardless of whether they were monomorphic or polymorphic in Kearney's bluestar (Table 2). The 19 initial primer pairs were narrowed to the 12 loci reported here that were polymorphic in a least one *Amsonia* species. The remainder were either monomorphic in all species or did not robustly and specifically amplify the locus.

Acknowledgements

References

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, **19**, 11–15.

McLaughlin SP (1982) A Revision of the Southwestern Species of *Amsonia* (Apocynaceae). *Annals of the Missouri Botanical Gardens*, **69**, 336–350.

Meredith EP, May B (2002) Microsatellite loci in Lahontan tui chub, *Gila bicolor obesa*, and their utilization in other chub species. *Molecular Ecology Notes*, **2**, 156–158.

Raymond M, Rousset F (1995) genepop 3.3, population genetics software for exact tests and ecomenicism. *Journal of Heredity*, **86**, 248–249.

Rodzen JR, Agresti JJ, Tranah GJ, May BP (1998) Agarose overlays allow simplified staining of polyacrylamide gels. *Biotechniques*, **25**, 584.

Welsh AB, Blumberg M, May B (2003) Identification of microsatellite loci in lake sturgeon, *Acipenser fulvescens*, and their variability in green sturgeon, A. medirostris. *Molecular Ecology Notes*, **3**, 47–55.

Woodson RE Jr (1928) Studies in the Apocynaceae. III A monograph of the genus *Amsonia*. *Annals of the Missouri Botanical Garden*, **15**, 379–428.