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Characterization of microsatellite loci in *Yucca brevifolia* (Agavaceae) and cross-amplification in related species[1]

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- *Premise of the study:* Microsatellite primers were characterized in *Yucca brevifolia* for use in population genetic studies and, particularly, analyses of gene flow between varieties.
- *Methods and Results:* We characterized 12 microsatellite loci polymorphic in *Yucca brevifolia* by screening primers that were developed using an SSR-enriched library or which were previously described in *Yucca filamentosa*. Genetic analysis of four populations resulted in the mean number of alleles per locus ranging from 10.25 to 14.58 and mean expected heterozygosity from 0.78 to 088. Cross-amplification of all 12 loci was attempted in six additional yucca species.
- *Conclusions:* These loci should prove useful for population genetic research in *Yucca brevifolia,* and cross-amplification of these loci in related species suggests that they may be useful in studies of hybridization and introgression between species.

Key words: gene flow; genetic variation; hybridization; microsatellite; *Yucca brevifolia.*

Within obligate pollination mutualism, the reliance of plants on specialized pollinators provides a mechanism of reproductive isolation between species (Fulton and Hodges, 1999). The relationship between yuccas and yucca moths is the archetypical example of an obligate pollination mutualism: yuccas are exclusively pollinated by yucca moths, while the moths oviposit only in yucca flowers. Each moth is typically specialized on a single host (Pellmyr, 2003), suggesting that pollinator specialization may prevent hybridization between *Yucca* species. However, recent work suggests that gene flow among species may be more common than was previously thought. Comparisons of chloroplast sequence data with AFLP markers suggest widespread introgression among species (Smith et al., 2008). Similarly, recent studies of the Joshua tree (*Yucca brevifolia* Engelm.) reveal that although there are two morphologically distinct varieties (var. *brevifolia* and var. *jaegeriana* McKelvey), each pollinated by a distinct species of moth (Godsoe et al., 2008), where the varieties occur in sympatry the two moths visit both tree types indiscriminately, presenting the opportunity for gene flow between varieties (Smith et al., 2009).

These results highlight the need for nuclear markers to measure pollinator-mediated gene flow among species. Here we describe 12 polymorphic microsatellite loci developed for *Y. brevifolia*. We also document their successful amplification in six additional species from within different subsections of the genus.

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METHODS AND RESULTS

An enriched library was made 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 $(GT)_{13}$, $(CT)_{13}$, $(GTAT)_7$ and $(GATA)_7$ oligonucleotide repeats (Gautschi et al., 2000). Of the 528 recombinant colonies screened, 195 gave a positive signal after hybridization. Plasmids from 80 positive clones were sequenced, and primers were designed for 21 microsatellite inserts. Of these, 13 loci were tested for polymorphism in *Yucca brevifolia*. Additionally, we tested three microsatellite markers developed for *Yucca filamentosa* L. (Sklaney et al., 2009).

Polymorphism was evaluated at the 16 loci in two populations of *Y. brevifolia* var. *brevifolia* (N = 21 and 31) and two populations *of Y. brevifolia* var. *jaegeriana* (N = 27 and 34). PCR amplification was performed in three 8-μL multiplex reactions with the QIAGEN multiplex PCR kit (QIAGEN, Valencia, California, USA). Each reaction contained 3.5 μL QIAGEN Multiplex PCR Master Mix, 0.7 μL Q-solution, 0.04–0.31 μM of each primer (see Table 1 for primer-specific concentrations; forward primers were labeled with 6-FAM, VIC, NED, or PET; Perkin Elmer, Applied Biosystems, Foster City, California, USA) and 20 ng DNA template. The thermal profile consisted of an initial denaturing step of 95°C for 15 min, 14 cycles of 94°C for 30 s, T_{A1} – 0.3°C per cycle for 90 s, and 72°C for 60 s, then 21 cycles of 94°C for 30 s, T_{A2} for 90 s, and 72°C for 60 s, and a final elongation step of 60°C for 30 min. Amplified DNA was genotyped on an ABI 3130 genetic analyzer (Perkin Elmer, Applied Biosystems) and alleles were scored using Genemarker v. 4.0 (SoftGenetics LLC, State College, Pennsylvania, USA). Although all 16 microsatellites were polymorphic, 11 of the 13 *Y. brevifolia* and 1 of the 3 *Y. filamentosa* markers provided allelic patterns suitable for downstream analysis. In two markers, Yucbre08 and Yucbre22, successive alleles often differed in length by a single base pair, despite these markers' di-nucleotide repeat motifs. These single base pair differences were confirmed via sequencing. Specifically, in Yucbre08, single base pair differences result from guanine insertions within the repeat motif. In Yucbre22, indels at multiple sites contribute to single base pair differences between alleles.

Allelic richness, heterozygosity, and deviations from Hardy–Weinberg equilibrium (HWE) were calculated for each of the four populations with the use of Arlequin v.3.5 (Excoffier et al., 2005) (Table 2). In some cases, deviations from HWE may reflect the presence of null alleles, as is likely in Yucbre05.

TABLE 1. Characterization of 12 polymorphic microsatellite markers in *Y. brevifolia*. Listed for each locus are the forward and reverse sequences of each primer pair, repeat motif, observed size range, first and second annealing temperatures used in the PCR thermal profile, the primer concentration used in the PCR protocol, and the GenBank accession number. The asterisk denotes the primer originally developed for *Y. filamentosa*.

Locus	Primer sequence	Repeat Motif	Size Range	T_{A1}/T_{A2}	primer conc.	GenbankID
Yucbre01	F: GCATGTGAACGCACATACCC R: AAGCTTGAATGGGAAGGATTAAC	$(CA)_{11}(GA)_{24}$	137–197	59/53	0.08 μM	HQ456641
Yucbre04	F: GCGCATTTTTGTTATTCTATGC R: TCAGCAGCAACCGACAATAG	$(CT)_6AT(CT)_7(GT)_{19}$	130–181	53/49	0.08 μM	HQ456642
Yucbre05	F: CAACACCAAATTCAAAATAACACC R: GGCCTAGATTTTGACACATCC	$(AC)_{18}GC(AC)_3(AT)_6$	111–173	59/53	0.15 μM	HQ456643
Yucbre06	F: CCAAGGTCCTTCGCTTCTATG R: TCCATGCTCAAATCCTTCTG	$(AG)_{23}$	204–260	53/49	0.08 μM	HQ456644
Yucbre08	F: ATTTGAGGGGGAAGGATTTG R: ACCGGCTACAACAGATGACC	$(GT)_{14}GG(GT)_3$	123–186	59/53	0.06 μM	HQ456650
Yucbre12	F: AACTCCCGTGTTTTGGTGTG R: AACTCTACTGCCATGTATGTACGC	$(TACA)_7$	101–136	53/49	0.04 μM	HQ456645
Yucbre13	F: AACAGAGAAGAGTGGGAGTTCG R: GATCAAAATCAAAAAGAAGACTTG	$(TG)_{17}(AG)_{14}$	165–233	53/49	0.13 μM	HQ456646
Yucbre15	F: TTCAATGATGAGCCAGATGC R: TGTTGCACAACAATGAAAGTG	$(CT)_{12}(CA)_{13}$	169–215	59/53	0.14 μM	HQ456647
Yucbre20	F: AAAATCACATAAATTGCATGATGG R: TGCAAAAGTCAATCCCAAGG	$(CT)_{16}AACT(GT)_{22}$	189–261	59/53	0.31 μM	HQ456651
Yucbre21	F: CACCCCAAGTCACTGTGC R: GGCTAATCTAGGGTTTAAAAGAGG	$(TC)_{16}$	085–131	59/53	0.04 μM	HQ456648
Yucbre22	F: AGCATTTGTTGTGTGCATCC R: GAGAGTTGTTGGCTCGTTGC	$(TC)_{26}$	121–217	59/53	0.06 μM	HQ456649
Msat-36*	F: TACCCGTTCTTGCGGATAGT R: GCTGAGTTCATCGTCGTCCT	$(CT)_9$	149–181	59/53	0.06 μM	EU867786

TABLE 2. Results of initial microsatellite marker screening in four populations of Y. brevifolia. Statistics are shown for two populations of *Y. brevifolia* var. *brevifolia* (Saddleback: 34°39′N, 117°47′W and Montezuma: 37°44′N, 117°23′W) and two populations of *Y. brevifolia* var. *jaegeriana* (Hancock: 37°27′N, 115°20′W and Lee Canyon: 36°24′N, 115°34′W). Parentheses denote the number of individuals sampled in each population. For each locus the number of alleles (*Na*), observed heterozygosity (H_o), expected heterozygosity (H_e) and deviations from Hardy–Weinberg equilibrium (HWE) are shown. Bold values represent significant deviations from Hardy–Weinberg equilibrium after B-Y FDR correction for multiple comparisons.

	Y. brevifolia var. *brevifolia*								*Y. brevifolia* var. *jaegeriana*							
	Saddleback (*N* = 21)				Montezuma (*N* = 31)				Hancock (*N* = 27)				Lee Canyon (*N* = 34)			
Locus	*Na*	H_o	H_e	HWE	*Na*	H_o	H_e	HWE	*Na*	H_o	H_e	HWE	*Na*	H_o	H_e	HWE
Msat-36	8	0.905	0.844	0.7828	5	0.516	0.652	0.0408	6	0.593	0.770	0.2445	10	0.882	0.777	0.7901
Yucbre01	17	0.810	0.943	0.0167	14	0.968	0.914	0.9081	15	0.963	0.916	0.6933	18	0.882	0.947	0.5476
Yucbre04	14	0.857	0.901	0.5654	10	0.839	0.820	0.1199	12	0.815	0.897	0.6667	16	0.824	0.887	0.0500
Yucbre05	12	0.524	0.900	**0.0000**	15	0.742	0.787	0.0869	11	0.296	0.857	**0.0000**	19	0.529	0.943	**0.0000**
Yucbre06	21	0.952	0.958	0.5045	18	1.000	0.929	0.4547	15	0.852	0.890	0.1278	20	0.941	0.946	0.7453
Yucbre08	11	0.762	0.876	0.4690	11	0.806	0.788	0.6356	9	0.333	0.775	**0.0000**	9	0.706	0.813	0.1879
Yucbre12	4	0.333	0.598	0.0167	3	0.452	0.436	1.0000	4	0.593	0.629	0.6557	5	0.588	0.693	0.0987
Yucbre13	13	0.810	0.905	0.1738	6	0.710	0.796	0.4201	11	0.815	0.842	0.1236	15	0.647	0.914	**0.0025**
Yucbre15	13	0.714	0.892	0.0318	9	0.806	0.814	0.2611	11	0.556	0.861	**0.0011**	13	0.794	0.842	0.8482
Yucbre20	15	0.952	0.912	0.8791	12	0.839	0.818	0.9917	10	0.815	0.893	0.4617	14	0.941	0.885	0.6286
Yucbre21	12	0.667	0.922	**0.0028**	7	0.806	0.748	0.7512	10	0.556	0.592	0.1826	13	0.765	0.889	0.1083
Yucbre22	19	0.905	0.937	0.5347	13	0.871	0.901	0.3883	19	0.889	0.928	0.3568	23	0.824	0.936	0.1333

Additionally, amplification at these 12 loci was attempted for three species in the subdivision Chaenocarpa (*Y. elata* Engelm., *Y. glauca* Nutall, *Y. rostrata* Engelm.) and three species in the subdivision Sarcocarpa (*Y. baccata* Torrey, *Y. schidigera* Roexl, *Y. shotti* Engelm.). With the exception of Yucbre05, all markers were successfully amplified in at least one of the six species tested (see Appendix 1).

CONCLUSIONS

The high levels of allelic richness and heterozygosity shown in the microsatellite markers described here should prove useful for a variety of population genetic measures. These markers have sufficient power to detect ecologically relevant patterns of genetic variation, including gene flow between varieties, which is integral to understanding hybridization and speciation in this plant-pollinator system. Finally, cross-amplification of these loci in related species suggests that they may be broadly applicable across the genus *Yucca*.

LITERATURE CITED

ARMOUR, J. A., R. NEUMANN, S. GOBERT, AND A. J. JEFFREYS. 1994. Isolation of human simple repeat loci by hybridization selection. *Human Molecular Genetics* 3: 599–605.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* 1: 47–50.

Fulton, M., and S. A. Hodges. 1999. Floral isolation between Aquilegia formosa and Aquilegia pubescens. *Proceedings. Biological Sciences* 266: 2247–2252.

Gautschi, B., A. Widmer, and J. Koella. 2000. Isolation and characterization of microsatellite loci in the dice snake (*Natrix tessellata*). *Molecular Ecology* 9: 2191–2193.

Godsoe, W. K. W., J. B. Yoder, C. I. Smith, and O. Pellmyr. 2008. Coevolution and divergence in the Joshua tree/yucca moth mutualism. *American Naturalist* 171: 816–823.

Pellmyr, O. 2003. Yuccas, yucca moths and coevolution: A review. *Annals of the Missouri Botanical Garden* 90: 35–55.

Sklaney, M. R., D. M. Althoff, and K. A. Segraves. 2009. Characterization of polymorphic microsatellite loci in *Yucca filamentosa. Molecular Ecology Resources* 9: 894–896.

Smith, C. I., O. Pellmyr, D. M. Althoff, M. Balcázar-Lara, J. H. Leebens Mack, and K. A. Segraves. 2008. Pattern and timing of diversification in *Yucca* (Agavaceae): Specialized pollination does not escalate rates of diversification. *Proceedings. Biological Sciences* 275: 249–258.

Smith, C. I., J. B. Yoder, W. Godsoe, and O. Pellmyr. 2009. Host specificity and reproductive success of yucca moths (*Tegeticula* spp. Lepidoptera: Prodoxidae) mirror patterns of gene flow between host plant varieties of Joshua tree (*Yucca brevifolia*: Agavaceae). *Molecular Ecology* 18: 5218–5229.

Appendix 1. Amplification success of 12 microsatellite markers across *Yucca* species.

The cross-amplification of 12 microsatellite loci characterized here in other species of *Yucca*. Successful amplification (+) and failed amplification (-) are indicated.

	Chaenocarpa			Sarcocarpa		
	Y. elata	*Y. glauca*	*Y. rostrata*	*Y. baccata*	*Y. schidigera*	*Y. shottii*
Msat-36	—	+	+	+	+	+
Yucbre01	+	—	+	+	+	+
Yucbre04	+	+	+	+	+	+
Yucbre05	—	—	—	—	—	—
Yucbre06	+	+	+	+	+	+
Yucbre08	+	+	+	+	+	+
Yucbre12	+	+	+	+	+	+
Yucbre13	+	+	—	+	+	+
Yucbre15	—	+	—	+	—	+
Yucbre20	+	+	+	+	+	+
Yucbre21	—	—	—	+	+	+
Yucbre22	+	+	+	+	+	+