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TECHNICAL NOTE

Isolation of microsatellite loci from *Rhamnus pirifolia*

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Abstract *Rhamnus pirifolia* Greene (Rhamnaceae), the island redberry, is a small evergreen tree endemic to the California Channel Islands and Guadalupe Island, Mexico. Nine polymorphic microsatellite loci were isolated from the taxon and were screened for variability in populations from three California Channel Islands. Moderate levels of variability were observed, with mean numbers of alleles per locus ranging from 1.3 to 4.7. The mean observed and expected heterozygosities ranged from 0.01 to 0.53 and 0.01 to 0.56, respectively. These new loci will be useful in conservation genetic and evolutionary studies within *Rhamnus*.

Keywords California Channel Islands · Conservation genetics · *Rhamnus* · Microsatellite

Rhamnus pirifolia (Rhamnaceae) is an island endemic species originally described from five of the eight California Channel Islands (and currently extant on four of them) and Guadalupe Island, Mexico (Sawyer 1993, Schoenherr et al. 1999, Wallace 1985). This species is associated with island chaparral, among the most ancient and unique California Island plant communities (Junak et al. 2007). *Rhamnus pirifolia* has been impacted by coastal development, military training, and introduced herbivores; although population sizes range from 1 to more than 50 individuals, San Clemente has fewer than 80 plants (Helenurm, unpubl. data) and Guadalupe Island appears to have fewer than 10 (Moran, 1996). The genetic effects of small population size warrants further study. Additionally, its distribution on the four largest California Channel Islands is well suited to inter-island studies of isolation and divergence. Here we report the characterization of nine microsatellite loci useful for conservation genetic and evolutionary studies within *Rhamnus pirifolia.*

Genomic DNA was isolated from leaf tissue using the DNeasy Plant Mini Kit (Qiagen). A microsatellite library was constructed from pooled DNA sampled from several San Clemente Island individuals. Isolation of microsatellite loci was performed following the subtractive hybridization method of Hamilton et al. (1999) with some modifications. Digested DNA was enriched for eight oligonucleotide repeats $(AC)_{15}$, $(AG)_{15}$, $(AT)_{15}$, $(CG)_{15}$, $(CCG)_{10}$, $(AAC)_{10}$, $(AGG)_{10}$, and $(CAC)_{10}$. Fragments were cloned using pBluescript II SK- Phagemid vector and the XL1-Blue MRF′ bacterial host strain (Stratagene). Color-positive clones were screened for microsatellite regions using a membrane based 'dot blot' method (Glenn and Schable, 2002) and the Phototope chemiluminescent detection system (New England Biolabs). A total of 112 positive clones were screened for insert size by PCR using an MJ Research PTC-200. The 25 µl reactions contained 1 µl template DNA, 0.8 µM each of primers T3 and T7 (Integrated DNA Technologies), 1× Thermopol Reaction Buffer (New England Biolabs), 200 µM of each dNTP, and 0.2 units of GoTaq Flexi DNA polymerase (Promega). Clones that exhibited a single amplified band of 400–1,000 bp were cleaned using a PEG precipitation procedure and sequenced using the T3 primer and BigDye Terminator

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Table 1 Primer sequences and diversity statistics for nine microsatellite loci isolated from *Rhamnus pirifolia*

Locus	GenBank accession number	Primer sequence (5′–3′)	5′ Tag	Label dye	Repeat motif	Allele size range	Island	Genetic diversity			
								N_A	H_O	H_E	HWE *P* value
RHPI_21	HQ593650	F-AATTCAAAACAAAACTAACTCATGC		6-FAM	$(GA)_9$	153–169	San Clemente	1	0.00	0.00	–
		R-TACTTCCCATCAACCCTCG	M13R				Santa Catalina	2	0.39	0.32	1.000
							Santa Cruz	2	0.44	0.44	1.000
							Mean	1.7	0.28	0.25	
RHPI_22	HQ593652	F-ATTAGAGCCTGGGAGGTCGT		PET	$(CTT)_{10}$	244–265	San Clemente	5	0.60	0.71	0.057
		R-CAGCAGCAGCCACAGAAATA	M13R				Santa Catalina	3	0.28	0.47	0.028
							Santa Cruz	3	0.22	0.23	0.378
							Mean	3.7	0.37	0.47	
RHPI_55	HQ593653	F-TGGGAGCAAGTGTTGATGAT	M13R	PET	$(AAG)_8$	189–195	San Clemente	1	0.00	0.00	–
		R-GCATGTATTCTATCTCACTCTCACA					Santa Catalina	1	0.00	0.00	–
							Santa Cruz	2	0.03	0.03	1.000
							Mean	1.3	0.01	0.01	
RHPI_68	HQ593654	F-TGGACCATGCTACTCGTGC		6-FAM	$(CT)_{16}$	187–213	San Clemente	3	0.55	0.61	0.854
		R-CATGGCACACATGGCAAC	CAGT				Santa Catalina	5	0.67	0.70	0.425
							Santa Cruz	5	0.38	0.38	0.632
							Mean	4.3	0.53	0.56	
RHPI_86	HQ593655	F-GAGATTGGGAGACTGAGG	CAGT	VIC	$(GA)_8$	230–232	San Clemente	2	0.10	0.10	1.000
		R-ACGTGGAGGAGGAAGATGG					Santa Catalina	2	0.06	0.06	1.000
							Santa Cruz	2	0.34	0.48	0.141
							Mean	2	0.17	0.21	
RHPI_114	HQ593647	F-GAAGTGGTGGGAGAGTCG	CAGT	VIC	$(GAA)_7$	300–309	San Clemente	3	0.50	0.42	0.186
		R-CCAATACTTGCTTCGCTTGC					Santa Catalina	2	0.33	0.41	0.555
							Santa Cruz	3	0.47	0.55	0.526
							Mean	2.7	0.43	0.46	
RHPI_132	HQ593648	F-GGATCCCATCTACATTTACACACC		VIC	$(GA)_{11}$	159–181	San Clemente	5	0.70	0.76	0.058
		R-TTCTTGTCATGGGCTGCTG	CAGT				Santa Catalina	6	0.67	0.69	0.736
							Santa Cruz	3	0.06	0.06	1.000
							Mean	4.7	0.48	0.50	
RHPI_152	HQ593649	F-TATACGTGTCCATGCAACAAC		6-FAM	$(AG)_9$	264–268	San Clemente	3	0.05	0.10	0.025
		R-CAGCAGAAGCAGAACTCAC	CAGT				Santa Catalina	1	0.00	0.00	–
							Santa Cruz	2	0.56	0.41	0.070
							Mean	2	0.20	0.17	

Table 1 continued

Locus	GenBank accession number	Primer sequence (5′–3′)	5′ Tag	Label dye	Repeat motif	Allele size range	Island	Genetic diversity			
								N_A	H_O	H_E	HWE *P* value
RHPI_217	HQ593651	F-GCAATCCCATTCAACCATCAT	CAGT	VIC	$(CT)_{10}$	107–121	San Clemente	3	0.10	0.38	0.000
		R-CGGTAAAGACATTTTGGCTGA					Santa Catalina	6	0.72	0.69	0.861
							Santa Cruz	2	0.06	0.06	1.000
							Mean	3.7	0.29	0.38	

Shown are loci names, the GenBank accession numbers, the forward (F) and reverse (R) primer sequence, the 5′ tag used for incorporation of the fluorescent tag M13R (AGGAAACAGCTATGACCAT) or CAGT (ACAGTCGGGCGTCATCA), labeling dye used, repeat motif of the sequenced clone, allele size range in base pairs, the number of alleles (N_A), observed heterozygosity (H_O), and expected heterozygosity (H_E) determined as the mean value from one population sampled from each of three islands within the range of *Rhamnus pirifolia*, San Clemente Island ($N = 20$), Santa Catalina Island ($N = 18$) and Santa Cruz Island ($N = 32$), and *P* value associated with departure from Hardy–Weinberg Equlibrium (HWE)

version 3.1 Cycle Sequencing Kit (Applied Biosystems) in 1/8 volume reactions. Sequences were electrophoresed on an Avant 3100 Genetic Analyzer (Applied Biosystems). For inserts containing a microsatellite motif, the T7 primer was used to generate a complementary reverse sequence. All sequences were aligned using SEQUENCHER 4.1 (GeneCodes).

Of the 86 sequenced inserts, 37 contained a region of at least six repeat units, but only 26 proved suitable for primer design. Primers were designed using the program PRIMER 3 (Rozen and Skaletsky, 2000). One primer of each pair was designed with a common tag at the 5′ end following the procedure of Boutin-Ganache et al. (2001; Table 1). Two common tags were used: M13R (AGGAAACAGC TATGACCAT) and CAGT (ACAGTCGGGCGTCATCA). We chose nine primers that yielded consistent amplification products. Loci were amplified with a common tag containing one of three fluorescent dyes, 6-FAM, PET, or VIC (Applied Biosystems).

A sample population containing 18–32 individuals was selected from each of three islands (San Clemente, Santa Catalina, Santa Cruz) to evaluate variability in the isolated microsatellite loci. Microsatellite loci were amplified in 10 μl reactions using the Type It Microsatellite PCR Kit (Qiagen). When possible, multiplex PCR with 2 loci was used. Manufacturer protocols and thermal cycler programs were used for all amplifications. PCR products were diluted with water and mixed with Hi-Di formamide and LIZ 500 size standard (Applied Biosystems) before electrophoresis on an Avant 3100 Genetic Analyzer. Fragments were sized using GENEMAPPER (Applied Biosystems). The presence of null alleles and size scoring errors were assessed with Micro-Checker (Van Oosterhout et al. 2004). We calculated observed (H_O) and expected (H_E), and tested for deviation from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium using Genepop version 3.4 (Raymond and Rousset 1995, Rousset 2008).

All loci were found to be polymorphic in at least one population. Two loci exhibited potential null alleles or stuttering error in a single population. RHPI_22 had a significant lack of heterozygotes in the Santa Catalina Island population and RHPI_217 had a significant lack of individuals heterozygous for alleles differing by a single repeat in the San Clemente Island population. No loci exhibited a persistent pattern suggestive of null alleles or scoring error across multiple populations. The mean number of alleles per locus ranged from 1.3 to 4.7, with an average of 2.9 (Table 1). Observed and expected mean heterozygosities ranged from 0.01 to 0.53 and 0.01 to 0.56, respectively, with the Santa Catalina Island population exhibiting the highest levels of diversity. Three loci, RHPI_22, RHPI_152, and RHPI_217, showed significant ($P < 0.05$) deviations from HWE in a single population,

Santa Catalina, San Clemente and San Clemente, respectively. Additionally, the San Clemente population deviated significantly ($P = 0.027$) from HWE in global tests across all loci. Of the 36 global interlocus comparisons, no loci exhibited significant linkage disequilibrium. Three of the 108 intrapopulation comparisons exhibited significant linkage disequilibrium ($P < 0.05$). The observed linkage disequilibria are likely driven by population differentiation, as no pair of loci exhibited linkage disequilibrium within more than one population.

The markers described in this paper will be used to investigate the population structure and levels of genetic variability of San Clemente Island *R. pirifolia* and to infer intra- and inter-island patterns of gene flow and divergence within the taxon.

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References

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques 31:24–28

Glenn TC, Schable M (2002) Microsatellite isolation with dynabeads. 2002. Unpublished manual. Savannah River Ecology Laboratory, Aiken, South Carolina

Hamilton MB, Pincus EL, Di Fiore A, Fleischer RC (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques 27:500–507

Junak S, Knapp DA, Haller JR, Philbrick R, Schoenherr A, Keeler-Wolf T (2007) The California Channel Islands. In: Barbour MG, Keeler-Wolf K, Schoenherr A (eds) Terrestrial Vegetation of California, 3rd edn. University of California Press, Berkeley, California, pp 229–252

Moran R (1996) The Flora of Guadalupe Island. Mexico. California Academy of Sciences, San Francisco, CA

Raymond M, Rousset F (1995) Genepop (Version-1.2)—Population-Genetics Software for Exact Tests and Ecumenicism. J Hered 86:248–249

Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol Ecol Res 8:103–106

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Misener S, Krawetz SA (eds) Bioinformatics methods and protocols. Humana Press, Totowa, N.J

Sawyer JO (1993) Rhamnus. In: Hickman JC (ed) The Jepson manual: higher plants of California. University of California Press, Berkeley, California, pp 940–942

Schoenherr AA, Felmeth CR, Emerson MJ (1999) Natural History of the Islands of California. University of California Press, Berkeley, California

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Wallace GD (1985) Vascular plants of the Channel Islands of southern California and Guadalupe Island, Baja California, Mexico. Contributions in Science No. 365 Natural History Museum of Los Angeles County, Los Angeles, California