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Microsatellite primers for the narrowly endemic shrub *Eriogonum giganteum* (Polygonaceae)[1]

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- *Premise of the study:* Microsatellite primers were designed for *Eriogonum giganteum* var. *formosum*, an endemic shrub of San Clemente Island, to investigate population structure, genetic diversity, and demographic history.
- *Methods and Results:* Twelve polymorphic microsatellite loci were isolated from the California Channel Island endemic *Eriogonum* and were screened for variability. The primers amplified one to eight alleles in the target taxon. Many primers also amplified in conspecific and congeneric (*E. arborescens*, *E. fasciculatum*, *E. grande*, *E. latifolium*, and *E. parvifolium*) taxa and in the closely related *Chorizanthe valida*. The total number of alleles per locus for all taxa screened ranged from three to 24.
- *Conclusions:* These primers will be useful for conservation genetic and evolutionary studies within the California Channel Island endemic *Eriogonum*.

Key words: California Channel Islands; conservation genetics; *Eriogonum*; *Eriogonum giganteum* var. *formosum*; microsatellite; Polygonaceae.

Eriogonum giganteum S. Watson (Polygonaceae) is a shrub endemic to three of the four southern California Channel Islands. Three single island endemic varieties, *E. giganteum* var. *compactum* Dunkle (Santa Barbara Island), *E. giganteum* var. *giganteum* (Santa Catalina Island), and *E. giganteum* var. *formosum* K. Brandegee (San Clemente Island), are recognized, although most diagnostic morphological characters have overlapping values (Hickman, 1993; but note the larger fruits and longer perianth of *E. giganteum* var. *formosum*). Additionally, the species hybridizes with both the northern California Channel Island endemic *E. arborescens* Greene and with the mainland native *E. fasciculatum* Benth. in cultivation (Hickman, 1993).

The extent to which the distribution of *E. giganteum* on oceanic islands is driving genetic divergence is currently being studied. The limited morphological differentiation between varieties and the broad tolerance for hybridization with California Channel Island and mainland congeners suggest that genetic differentiation has been limited. However, the distinct morphology of the most isolated variety and the species' and varieties' endemicity suggest that the interisland and island-mainland water barriers are sufficient to disrupt gene flow and genetically isolate the taxa. Here we report the characterization of 12 microsatellite loci that will be used to determine the population structure and genetic diversity of *E. giganteum* var. *formosum*, to ascertain the degree of genetic differentiation among *E. giganteum* varieties, and to investigate the evolutionary patterns within and among *Eriogonum* Michx. species endemic to the California Channel Islands. Many of the loci also amplify for, and are variable within, additional mainland *Eriogonum* and *Chorizanthe* R. Br. ex Benth. taxa.

METHODS AND RESULTS

Genomic DNA was isolated from leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). Seven separate microsatellite libraries were constructed from pooled individuals of (a) *E. giganteum* var. *formosum* from San Clemente Island, (b) *E. giganteum* from Santa Barbara, Santa Catalina, and San Clemente islands, (c) *E. giganteum* var. *compactum* from Santa Barbara Island, (d) *E. arborescens* from Santa Cruz Island, (e) *E. arborescens* from Santa Cruz and Santa Rosa islands, and (f) *E. grande* Greene from Santa Cruz, San Clemente, and San Nicolas islands (two libraries). Isolation of microsatellite loci followed 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 the pBluescript II SK(−) Phagemid vector and the XL1-Blue MRF′ bacterial host strain (Stratagene, Cedar Creek, Texas, USA). Color-positive clones were screened for microsatellite regions using a membrane "dot blot" method (Glenn and Schable, 2002) and the Phototope-Star chemiluminescent detection system (New England Biolabs, Ipswich, Massachusetts, USA). A total of 698 positive clones were screened for insert size by PCR using an MJ Research PTC-200 thermal cycler (MJ Research, Waltham, Massachusetts, USA). The 25 μL reactions contained 1.5 μL template DNA, 0.8 μM each of primers T3 and T7 (Integrated DNA Technologies, Coralville, Iowa, USA), 1× Thermopol Reaction Buffer (New England Biolabs), 200 μM of each dNTP, and 0.2 U of GoTaq Flexi DNA Polymerase (Promega, Madison, Wisconsin, USA). Clones that exhibited a single amplified band of 300–1000 bp were cleaned using a PEG precipitation procedure and sequenced using the T3 primer and BigDye Terminator version

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TABLE 1. Characteristics of 12 microsatellite primers developed for *Eriogonum giganteum* var. *formosum*. Shown for each primer pair are the GenBank accession number of the sequenced clone, the forward (F) and reverse (R) primer sequences, the dye used to visualize the fragment during data collection, repeat motif, and size of the original fragment.

Primer	GenBank Accession No.	Primer sequence (5′–3′)	Dye	Repeat motif	Size (bp)
ERGR_43	JF999979	F: CCAACACTAACATCCAAATTCTATC R: AGCGCTTCAAAAGATGGTGG	PET	CTT_{12}	188
EGIC_82	JF999971	F: CAGCTGGGTTTGCATGTCC R: AAAGCAGCAAGACCTGTTATC	VIC	CA_{10}	154
ERAR_85	JF999968	F: AGTGGCACGTGTTGAAACC R: GTTGGGTGTCTTAGTGGCG	PET	CA_{8}	127
ERGI_94	JF999974	F: (CAGT)-GCATACCCTTTCCCATGCC R: GATGGGGTGGTGAGTGGAG	PET	CT_{13}	176
EGIC_95	JF999972	F: GCTCTCTCTCTCTCTCTCGC R: ATGGAGGTTGCTCAGTCGG	6-FAM	CT_{7}	187
EGIC_96	JF999973	F: TGACACGGCCTTTTCTTTGC R: AGAAGGCACATCCGTAGCG	PET	GA_{9}	214
ERGI_99	JF999975	F: AGCTCCCCATCTCTCTCTTC R: CTCTCTTCACGCTCTCTTGC	PET	GA_{23}	218
EGIC_110	JF999969	F: GTGTCACAAATGGGAAAGCAC R: TGGCAGATAGTTTGGTGGAA	6-FAM	GAA_{6}	171
EGIC_144	JF999970	F: CCGCTTTGCCCCTATCTTG R: GCCGCCAAACAGGTTACTC	VIC	CT_{18}	158
ERGR_162	JF999976	F: GACGAAAGGGAGACGGGAG R: TCTCATGGTGACATCAGTAACAAC	6-FAM	GA_{12}	183
ERAR_221	JF999967	F: CACCCTCCCTTCTCCTTCC R: (CAGT)-GTTCAAACCAACTGCAACCC	VIC	CT_{13}	201
ERGR_308	JF999978	F: CCCACACTCTCCAAACCAAT R: GGCAAAGAGGGTGAAAGAGA	VIC	CT_{19}	193

3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, California, USA) in 1/8 concentration 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, Ann Arbor, Michigan, USA).

Of the 537 sequenced inserts, 152 contained a region of at least eight repeat units, but only 67 proved suitable for primer design. Primers were designed for these using the programs Primer3 (Rozen and Skaletsky, 2000) and MSAT Commander (Faircloth, 2008). One primer of each pair was originally designed with a common tag at the 5′ end following the procedure of Boutin-Ganache et al. (2001). Two common tags were used: M13R (AGGAAACAGCTATGACCAT) and CAGT (ACAGTCGGGCGTCATCA). Thirty-one primer pairs appeared to yield consistent products and were amplified with the common tag, CAGT or M13R, containing one of three fluorescent dyes, 6-FAM, PET, or VIC (Applied Biosystems), or were redesigned without the common tag and individually labeled with one of the aforementioned dyes (Table 1). Preliminary sizing of the fluorescently labeled products revealed 12 primer pairs that consistently yielded amplicons of the expected size and banding pattern (i.e., one or two bands per individual varying by the repeat motif) in *Eriogonum giganteum* var. *formosum*.

Three sample populations (n = 28–32) of the focal taxon, one population of each conspecific taxon, and one population of the northern California Channel Island congeneric *E. arborescens* were initially selected to evaluate variability in the isolated loci. Cross-amplification was tested more broadly with small samples (n = 8–15) of other Channel Island and mainland congeners (*E. grande*,

TABLE 2. Results of initial primer screening in populations of *Eriogonum giganteum* and *E. arborescens*. Shown for each population and primer pair are number of alleles and observed heterozygosity.

	ERGIFO A (N = 32)[b]		ERGIFO B (N = 32)		ERGIFO C (N = 29)		ERGIGI (N = 28)		ERGICO (N = 29)		ERAR (N = 31)	
Primer[a]	N_a	H_o	N_a	H_o	N_a	H_o	N_a	H_o	N_a	H_o	N_a	H_o
Ergr_43 (166; 163–175)	1	0.000	1	0.000	1	0.000	—	—	—	—	—	—
Egic_82 (158; 144–172)	1	0.000	1	0.000	1	0.000	2	0.321	2	0.069	2	0.419
Erar_85 (131; 115–137)	1	0.000	1	0.000	1	0.000	2	0.071	1	0.000	3	0.129
Ergi_94 (154–182; —)	5	0.367	4	0.531	3	0.038	—	—	—	—	—	—
Egic_95 (185; 185–189)	1	0.000	1	0.000	1	0.000	2	0.043	1	0.000	—	—
Egic_96 (210–214; 204–214)	2	0.031	3	0.156	3	0.034	3	0.179	3	0.241	1	0.000
Ergi_99 (216–240; 220–286)	3	0.594	3	0.500	3	0.231	8	0.643	7	0.724	10	0.387
Egic_110 (168; 156–177)	1	0.000	1	0.000	1	0.000	1	0.000	2	0.138	1	0.000
Egic_144 (162–182; 138–158)	2	0.133	4	0.667	2	0.517	1	0.000	4	0.655	—	—
Ergr_162 (207–211; 181–187)	1	0.000	3	0.259	1	0.000	—	—	—	—	—	—
Erar_221 (195–203; 201–223)	1	0.000	2	0.125	1	0.000	—	—	—	—	5	0.355
Ergr_308 (179–195; 179–193)	4	0.519	5	0.419	5	0.409	6	0.590	—	—	4	0.533

Note: — = amplification unsuccessful; ERGICO = *E. giganteum* var. *compactum*; ERGIFO = *E. giganteum*; var. *formosum* (three populations, see Appendix 1); ERGIGI = *E. giganteum* var. *giganteum*; ERAR = *E. arborescens*; H_o = observed heterozygosity; N_a = number of alleles.

[a] Included in parentheses are: allele range in *E. giganteum* var. *formosum*; allele range in all other taxa screened.

[b] The sample size for each population is shown in parentheses.

TABLE 3. Results of initial primer screening in additional *Eriogonum* populations and *Chorizanthe valida*. Shown for each taxon and primer pair are number of alleles and observed heterozygosity.

	ERGRGR (N = 8)[b]		ERGRRU (N = 8)		ERGRTE (N = 8)		ERGRTI (N = 8)		ERFA (N = 8)		ERLA (N = 8)		ERPA (N = 8)		CHVA (N = 15)	
Primer[a]	N_a	H_o	N_a	H_o	N_a	H_o	N_a	H_o	N_a	H_o	N_a	H_o	N_a	H_o	N_a	H_o
Ergr_43 (166; 163–175)	4	0.500	2	0.000	3	0.500	2	0.143	2	0.429	1	0.000	3	0.375	—	—
Egic_82 (158; 144–172)	2	0.375	2	0.143	1	0.000	2	0.625	5	0.750	2	0.250	2	0.375	3	0.133
Erar_85 (131; 115–137)	2	0.125	2	0.250	2	0.125	1	0.000	3	0.500	1	0.000	1	0.000	3	0.286
Ergi_94 (154–182; —)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Egic_95 (185; 185–189)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Egic_96 (210–214; 204–214)	3	0.500	2	0.200	3	0.167	3	0.143	2	0.000	2	0.200	1	0.000	5	0.286
Ergi_99 (216–240; 220–286)	—	—	—	—	—	—	—	—	3	0.143	—	—	—	—	—	—
Egic_110 (168; 156–177)	3	0.375	2	0.250	1	0.000	1	0.000	1	0.000	1	0.000	1	0.000	2	0.067
Egic_144 (162–182; 138–158)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ergr_162 (207–211; 181–187)	2	0.125	2	0.600	1	0.000	1	0.000	—	—	—	—	—	—	—	—
Erar_221 (195–203; 201–223)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ergr_308 (179–195; 179–193)	1	0.000	1	0.000	2	0.125	4	0.875	4	0.750	3	0.250	2	0.142	5	0.333

Note: — = amplification unsuccessful; CHVA = *Chorizanthe valida*; ERFA = *E. fasciculatum*; ERGRGR = *E. grande* var. *grande*; ERGRRU = *E. grande* var. *rubescens*; ERGRTE = *E. grande* var. *testudinum*; ERGRTI = *E. grande* var. *timorum*; ERLA = *E. latifolium*; ERPA = *E. parvifolium*; H_o = observed heterozygosity; N_a = number of alleles.

[a] Included in parentheses are: allele range in *E. giganteum* var. *formosum*; allele range in all other taxa screened.

[b] The sample size for each population is shown in parentheses.

E. fasciculatum, *E. latifolium* Sm., and *E. parvifolium* Sm.) and with *Chorizanthe valida* S. Watson. Microsatellite loci were amplified in 10 µL reactions with the Type-it Microsatellite PCR Kit (QIAGEN, Germantown, Maryland, USA). All amplifications followed manufacturer protocols and suggested thermal cycler programs (30 cycles with a 57°C annealing temperature, followed by eight cycles with a 53°C annealing temperature). PCR products were diluted with water and mixed with Hi-Di formamide and LIZ 500 size standard (Applied Biosystems) before electrophoresis on an Applied Biosystems 3500 Genetic Analyzer. Fragments were sized using GeneMarker software (Softgenetics, State College, Pennsylvania, USA).

Eleven of the 12 loci screened were polymorphic in at least one *E. giganteum* population, although observed heterozygosities were low (0.00–0.75; Tables 2, 3), particularly in *E. giganteum* var. *compactum* and *E. giganteum* var. *formosum*. All but one locus consistently amplified in other taxa, and many loci amplified in all additional taxa screened. Preliminary analyses with STRUCTURE (Pritchard et al., 2000; Falush et al., 2003; Hubisz et al., 2009) suggest that the suite of loci is sufficiently informative to capture infra- and intervarietal genetic differentiation (data not shown).

CONCLUSIONS

We found that the microsatellite markers reported here are variable and informative within and among *E. giganteum* varieties. These markers will be used to investigate the population genetic structure and levels of genetic variability of the San Clemente Island endemic *E. giganteum* var. *formosum* and, more broadly, to infer intra- and interisland patterns of gene flow and divergence within *E. giganteum*. Additionally, many markers successfully amplified in, and were variable for, mainland and Channel Island endemic congenerics and for the federally endangered species *Chorizanthe valida*. The markers will, therefore, also be used in population and conservation genetic studies of closely related taxa.

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APPENDIX 1. Voucher information for this study. Information presented: taxon, collection locale (GPS coordinates*), voucher specimen**, herbarium.

Chorizanthe valida S. Watson: USA, CA, Point Reyes National Seashore.

Eriogonum arborescens Greene: USA, CA, Santa Cruz Island (34.07315°N, 119.91799°W), *MEM208*, GREE20421.

Eriogonum fasciculatum Benth.: USA, CA, Santa Paula (34.43220°N, 119.12420°W), *MEM217*, GREE20420.

Eriogonum giganteum S. Watson **var. *compactum*** Dunkle: USA, CA, Santa Barbara Island (33.46922°N, 119.03945°W). ***Eriogonum giganteum*** S. Watson **var. *formosum*** K. Brandegee: USA, CA, San Clemente Island (A, 32.96617°N, 118.52810°W; B, 32.90470°N, 118.47166°W; C, 32.88483°N, 118.48959°W). ***Eriogonum giganteum*** S. Watson **var. *giganteum***: USA, CA, Santa Catalina Island (33.32353°N, 118.31443°W).

Eriogonum grande Greene **var. *grande***: USA, CA, San Clemente Island (32.89255°N, 118.49420°W). ***Eriogonum grande*** Greene **var. *rubescens*** (Greene) Munz: USA, CA, Santa Cruz Island (34.01603°N, 119.87764°W), *MEM207*, GREE20424. ***Eriogonum grande*** Greene **var. *testudinum*** Reveal: MX, BC, Punta Banda (ca. 31.74501°N, 116.74139°W), *EK 591*, RSA/POM. ***Eriogonum grande*** Greene **var. *timorum*** Reveal: USA, CA, San Nicolas Island (33.22919°N, 119.43575°W).

Eriogonum latifolium Sm.: USA, CA, Santa Cruz County (37.03919°N, 122.22772°W), *MEM252*, GREE20423.

Eriogonum parvifolium Sm.: USA, CA, Los Angeles (33.94170°N, 118.43561°W), *MEM218*, GREE20422.

Note: BC = Baja California; CA = California; MX = Mexico.

* GPS coordinates are not provided for the federally endangered *Chorizanthe valida.*

** Voucher specimens unavailable for tissue collected from plants growing on lands managed by the Catalina Island Conservancy, the United States Department of Defense, or the United States National Park Service.