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Authors: López-Villalobos, Adriana, Samis, Karen E., and Eckert, Christopher G.

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PRIMER NOTE

MICROSATELLITE PRIMERS FOR *CAMISSONIOPSIS CHEIRANTHIFOLIA* (ONAGRACEAE) AND CROSS-AMPLIFICATION IN RELATED SPECIES[1]

ADRIANA LÓPEZ-VILLALOBOS[2,4], KAREN E. SAMIS[3], AND CHRISTOPHER G. ECKERT[2]

[2]Department of Biology, Queen's University, Kingston, K7L 3N6 Ontario, Canada; and [3]Department of Biology, University of Prince Edward Island, Charlottetown, C1A 4P3 Prince Edward Island, Canada

- *Premise of the study:* We developed 24 nuclear microsatellite primers from an enriched genomic library for the Pacific coastal dune endemic *Camissoniopsis cheiranthifolia* to study the consequences of mating system differentiation, the genetics of species' range limits, and hybridization with its closest sister taxon, *C. bistorta.*
- *Methods and Results:* Twenty-four primer pairs were developed and characterized in four populations of *C. cheiranthifolia* and one population of *C. bistorta*. We also tested eight additional taxa for cross-amplification. The average number of alleles per locus per species was 4.3 and 6.0, respectively. The number of loci that amplified and were variable within the eight related taxa ranged from six to 17.
- *Conclusions:* These markers will be useful in studying mating system evolution, the genetic structure of species' ranges, hybridization, and the provenance of material used for habitat restoration in *C. cheiranthifolia*, *C. bistorta*, and related species.

Key words: *Camissoniopsis bistorta*; *Camissoniopsis cheiranthifolia*; hybridization; microsatellites; outcrossing; self-fertilization.

Camissoniopsis cheiranthifolia (Hornem. ex Spreng.) W. L. Wagner & Hoch (Onagraceae) is a diploid, bee-pollinated, short-lived perennial endemic to the Pacific coastal dunes of Baja California, California, and Oregon (Raven, 1969; Wagner et al., 2007). Being restricted to coastal dunes, it is continuously distributed along a near-linear, easily accessed geographic range, providing opportunities for studying the ecology and evolution of geographic range limits (Samis and Eckert, 2007, 2009). This species also exhibits striking variation in floral traits and the relative importance of outcrossing vs. self-fertilization, providing opportunities to investigate the evolution of mating systems (Eckert et al., 2006; Button et al., 2012). Dart et al. (2012) showed that populations in southern California are large-flowered (LF), predominantly outcrossing, and either largely self-incompatible (SI) or self-compatible (SC). Populations in Baja California toward the southern range limit, on the Channel Islands off California and north of Point Conception, California, to the northern range limit in southern Oregon are small-flowered (SF), SC, and predominantly selfing. The proportion of seeds outcrossed estimated at the population level from the segregation of allozyme polymorphism in progeny arrays ranged from 0.0–1.0 and correlated positively with flower size. Lineages within *Camissoniopsis* W. L. Wagner & Hoch and closely related *Eulobus* Nutt. ex Torr. & A. Gray and *Camissonia* Link appear to have undergone speciation via polyploidization involving hybridization (Raven, 1969; Wagner et al., 2007). In *Camissoniopsis*, five of 14 species are polyploid, predominantly selfing, and were likely derived through hybridization. *Camissoniopsis cheiranthifolia* and *C. bistorta* (Nutt. ex Torr. & A. Gray) W. L. Wagner & Hoch are the only two species that include outcrossing populations. Throughout the genus, species' ranges frequently overlap, and ongoing hybridization may be maintaining high morphological variation within and low differentiation among species. We developed microsatellite markers for *C. cheiranthifolia* that would cross-amplify in related taxa to better investigate mating system evolution, the genetic structure of geographic ranges, and the ecology and genetics of hybridization.

METHODS AND RESULTS

A microsatellite-enriched genomic library was developed following Glenn and Schable (2005) and Hamilton et al. (1999). Using silica-dried leaf tissue from one plant from each of two populations (Appendix 1), total DNA was isolated using cetyltrimethylammonium bromide (CTAB) extraction (Doyle and Doyle, 1987). We digested 5 µg of pooled DNA at 37°C overnight with *Alu*I + *Hae*III + *Rsa*I restriction enzymes. Digested DNA was dephosphorylated using 0.01 unit calf intestinal alkaline phosphatase per picomole ends of DNA at 50°C for 1 h, purified using an equal volume of 25 : 24 : 1 phenol : chloroform : isoamyl alcohol, precipitated using 2.5 volumes of cold 100% ethanol

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[4]Author for correspondence: lopezv.adriana@gmail.com

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TABLE 1. Characteristics of 24 microsatellite primer pairs developed for *Camissoniopsis cheiranthifolia*.

Locus	Primer sequences (5′–3′)	Repeat motif	T_a (°C)	Allele size range (bp)[a]	Multiplexed[b]	GenBank accession no.
A18	F: TCCTGTTGTTGTCCTTTCTT	$(TC)_{28}$	55	212–225	single	Pr032165043
	R: CCTCGTACAAGGACATGG					
A31b[c]	F: GAAGCCCTTCAGAGGTTAAT	$(TC)_{10}$	55	220–242	single	Pr032165044
	R: TAACCTCCTGGTCTTTCAGA					
A31c[c]	F: TGCTAGCAGAAGCCCTTCAG	$(TC)_{10}$	55	174–211	single	Pr032165045
	R: GTGCCTGACCTATGATGTCG					
B11	F: CCTGAAAAATGGAAATTGTGC	$(GA)_9$	55	120–152	2plex1	Pr032165046
	R: TTCACAGGACAGGACTGGAC					
B34	F: CACATTCCTTCACATTTGGT	$(TC)_{10}$	57	237–253	2plex2	Pr032165047
	R: CTTCAAAGGACAACCCTTTT					
B59	F: TCCTAACCATGCCGACTCGT	$(TC)_{25}$	57	121–179	2plex5	Pr032165048
	R: ACAGCAACTTCCCTGCAATCA					
C110	F: AATCCGAACGCTAACCACAG	$(GA)_8$	57	194–210	2plex2	Pr032165049
	R: TCAACCTCGAATCCAAGTCC					
C133	F: TTTACTGTCTTTGGTGTCTG	$(GA)_{14}$	55	121–157	2plex4	Pr032165050
	R: GGCTGCTGAGGAGAAGAT					
C135b[c]	F: ACAGTGGTGGTTTCAATTTC	$(TC)_{12}$	57	131–149	3plex	Pr032165051
	R: CAAAGAGCGAAGAAGAAGAA					
C135c[c]	F: CCGCCTTCATCTGTACTCCA	$(TC)_{12}$	57	219–255	single	Pr032165052
	R: AGTGTATTGGCGATTTCAGG					
C18	F: CCTGGTGCTACTCCTATGTAT	$(GA)_{15}$	57	173–211	single	Pr032165053
	R: GCCTTTCCTTATTGCAATCGT					
C19	F: GAAAAAGGAGTTGGTGCAG	$(TC)_{14}$	57	222–316	3plex	Pr032165054
	R: CAAAGAGAAATGTGGCAAAC					
C32	F: TCTCTTCTTCCTTTCCTCCT	$(GA)_{14}$	55	189–217	single	Pr032165055
	R: CCTGAAATCCAGTGATCATA					
C42	F: CCTGAAATCCAGTGATCATA	$(TC)_{14}$	55	243–255	single	Pr032165056
	R: GCATAGGATACTGTGGGGTA					
C49	F: GACGGGCAATAGAGTTTACA	$(TG)_{12}$	57	196–214	3plex	Pr032165057
	R: TATAGACTGCCGGCTTTAAC					
C55	F: AAGGAGAGGACAGGCTGTTG	$(GA)_{14}$	57	123–155	single	Pr032165058
	R: GCAGATCACATACCTCTGCTT					
C66	F: TGCTTATAAGTGATGATGCCT	$(GA)_9GT(GA)_3$	57	209–247	single	Pr032165059
	R: CTGGTCCAAATTCCTCTGGT					
C67	F: GAAGTACGAGATGCAGAACG	$(TC)_{15}$	57	233–257	2plex3	Pr032165060
	R: GCATACCTCAGAACGCTTAG					
C89	F: TGAAATCATGCACCGGACTA	$(TA)_5(GA)_8$	57	196–212	2plex5	Pr032165061
	R: AAAGGATTCTTGTGAAGGAATGA					
D17	F: CCATGCATTATTTCCAACTC	$(TC)_{24}$	57	215–249	single	Pr032165062
	R: TCCTCTCACTTCGTGTTTTC					
E19b	F: CTTTTCAAAGGTGGGAGCAA	$(TC)_{24}$	57	205–247	single	Pr032165063
	R: GCCTGCAAATAATGCCATGT					
E30	F: CATTGCTGTGCTTCTGTTC	$(TC)_{17}$	55	180–218	2plex1	Pr032165064
	R: CTCTACTTGTGGCTGTGGAT					
E42	F: TGTCTCCTTCCTGTGTGTGG	$(GA)_{10}$	55	179–197	2plex4	Pr032165065
	R: AAAATCCTCCATCCCCTGTC					
E70	F: GATATGGCTTACAATGCAACG	$(TC)_{15}$	57	128–144	2plex3	Pr032165066
	R: GTGAAGCAGTGAACCAAGCA					

Note: T_a = annealing temperature.

[a] Range of fragment sizes including the M13 tag (5′-CACGACGTTGTAAAACGA-3′) attached to the forward primer.

[b] For genotyping, we used single primer pair reactions for 11 loci, one triplex reaction (loci C135b+C49+C19), and five duplex reactions (B11+E30, C110+B34, E70+C67, C133+E42, and B59+C89), adjusting the number of cycles in the PCR program for B59+C89 to 32 (Table 1).

[c] For two loci (A31b and C135b), we developed two additional primer pairs (A31c and C135c; see text for details).

and 3 M sodium acetate (NaOAc), and then resuspended in TE buffer (10 mM Tris [pH 8.0], 1 mM EDTA). DNA quality and size were evaluated on 1.5% agarose gels (fragments ranged from 200–1000 bp).

Digested DNA was ligated to 1 μM of SNX double-stranded linkers using T4 DNA ligase (Invitrogen, Burlington, Ontario, Canada) and 20 units *Xmn*I (New England Biolabs, Whitby, Ontario, Canada) overnight at 16°C. Linker ligation was tested using PCR amplification with SNX forward primer (5′-CTAAGGCCTTGCTAGCAGAAGC-3′) in a reaction with 1× buffer, 2.0 mM $MgCl_2$, 150 μM dNTPs (Roche Diagnostics, Laval, Quebec, Canada), 0.5 μM primer, 1 μg/μL bovine serum albumin (BSA), and 1 unit *Taq* polymerase (reagents from Invitrogen except dNTPs).

Linker-ligated DNA was hybridized to 3′ biotinylated $(AC)_{13}$ and $(AG)_{13}$ probes for 4 h at 70°C after 10 min at 95°C. Enriched DNA was captured using streptavidin beads (DynaBeads M-280 Streptavidin, Invitrogen) and verified with PCR as above. Approximately 20 ng/μL of amplified DNA was used in transformation with the TOPO TA Cloning Kit (Invitrogen) and grown on Luria-Bertani plates with 50 ng/mL ampicillin. About 350 colonies were screened for microsatellites using fluorescent DIG probes (Roche Diagnostics). For positive clones, insert sizes were estimated with PCR using M13 primers and verified on 1% agarose gels. DNA was extracted from 115 positive clones with appropriate insert sizes and PCR products were sequenced at Genome Quebec (McGill University, Montreal, Quebec, Canada) or Robarts Research Institute (University of Western Ontario, London, Ontario, Canada). Ninety-three of these clones contained a total of 90 unique microsatellite regions. Primer pairs were designed for the 32 clones that had both linkers, suitable flanking region at both ends, and a minimum of eight repeats. We used Primer3web version 4.0.0 (Koressaar and Remm, 2007;

Untergasser et al., 2012) and Amplifix 1.5.4 (http://crn2m.univ-mrs.fr/AmplifX) to design primer pairs optimized to contain 18–22 bases, 40–60% GC content, 50–60°C melting temperature, and yield 100–350 bp PCR products.

The forward primer of each pair was labeled with a D4 red–labeled M13 tail (5′-CACGACGTTGTAAAACGA-3′) (Sigma-Aldrich Canada, Oakville, Ontario, Canada). The number and the identity of samples used for an initial testing of each pair varied. We used one to seven DNA samples from five to 42 (mean = 30.6) populations covering the entire geographic range of *C. cheiranthifolia* and one to six DNA samples from three to 12 (mean = 9.7) populations of *C. bistorta* (Appendix 1). Each sample was genotyped twice in single-locus 5-µL PCR reactions containing 0.5 µL of DNA template (10 µg/µL), 2.5 µL of Multiplex PCR Master Mix (QIAGEN, Toronto, Ontario, Canada), 0.1 µL of each forward and reverse primers (10 µM), 1.1 µL of M13taq (1 µM; Sigma-Aldrich Canada), 0.2 µL of Q-Solution, and 0.5 µL of sterile double-distilled water. PCR involved 15 min of denaturation at 94°C, followed by 35 cycles of 20 s at 94°C, 30 s at 55°C or 57°C, and 40 s at 72°C, with a 10-min final extension at 72°C. PCR product was diluted with double-distilled water to a final volume of 15 µL, and fragments were sized using a GenomeLab GeXP with the CEQ 8000 Genetic Analysis System version 9.0 (Beckman Coulter, Mississauga, Ontario, Canada).

Of 32 primer pairs, 24 yielded variable fragments of expected size and two of these amplified within two other loci (Table 1). For these two loci (A31b and C135b), a second primer pair (A31c and C135c) was redesigned to improve consistency of amplification in some *C. cheiranthifolia* but mainly in *C. bistorta* populations. For each locus, we estimated the number of alleles (A), observed (H_o) and expected (H_e) heterozygosities in one LF-SI population, one LF-SC population, two SF-SC populations (southern and northern parts of the range), and one LF-SI *C. bistorta* population using GenAlEx version 6.5 (Peakall and Smouse, 2012). We did not test for deviations from Hardy–Weinberg equilibrium because all populations of *C. cheiranthifolia*, including LF-SI populations, can exhibit some self-fertilization (Dart et al., 2012), so that H_o is less than H_e in many cases reported below.

Within populations, A ranged from one to 12 across loci (mean = 4.3) and was highest in the LF-SI populations compared to the LF-SC population and the two SF-SC populations (Table 2). Using only 13 loci for which the same individuals were genotyped, we detected 130 alleles total, of which 56 were found only in *C. cheiranthifolia* (mean ±1 SE = 4.30 ± 0.49 private alleles per locus) and 10 only in *C. bistorta* (0.77 ± 0.26 private alleles per locus), suggesting that these markers could be useful to detect hybridization between these species, although a broader sample is required to determined which are diagnostic. H_o and H_e were highly variable but predictable based on the mating system, as both were highest in the two LF-SI populations, lower in the mixed-mating LF-SC population, and lower still in the two SF-SC populations (Table 2), thereby verifying the potential of these markers for studying the genetic consequences of mating system differentiation. Although cross-amplification often failed in samples from the eight related taxa, there were many loci at which amplification was successful (Appendix 1, Table 3). Of the 24 loci developed for *C. cheiranthifolia*, 17 were tested in *C. micrantha* (Hornem. ex Spreng.) W. L. Wagner & Hoch, *C. lewisii* (P. H. Raven) W. L. Wagner & Hoch, *Eulobus angelorum* (S. Watson) W. L. Wagner & Hoch, *E. californicus* Nutt. ex Torr. & A. Gray, and *E. crassifolius* (Greene) W. L. Wagner & Hoch, and successful amplification occurred for 17, 15, nine, and nine loci, respectively. Dick et al. (2014) tested 16 of these 24 loci in the serpentine endemic *Camissonia benitensis* P. H. Raven and its two widespread congeners *C. strigulosa* (Fisch. & C. A. Mey.) P. H. Raven and *C. contorta* (Douglas) Kearney and found six variable loci, which they used to quantify patterns of genetic diversity.

CONCLUSIONS

All 24 microsatellite loci were variable in *C. cheiranthifolia* and *C. bistorta*, and a number of them also amplified in eight

TABLE 2. Estimation of population genetic parameters for 21 microsatellite loci in four *Camissoniopsis cheiranthifolia* populations representing each geographic region and mating type, plus one population of the sister species *C. bistorta*. Population codes (in parentheses) are provided in Appendix 1.[a]

	C. cheiranthifolia																*C. bistorta*			
	LF-SI (CBF)				LF-SC (CCO)				Southern SF-SC (BES)				Northern SF-SC (CMC)				LF-SI (CCU)			
Locus	n	A	H_o	H_e	n	A	H_o	H_e	n	A	H_o	H_e	n	A	H_o	H_e	n	A	H_o	H_e
A18	8	5	0.37	0.77	12	3	0.33	0.50	15	4	0.47	0.54	16	2	0.06	0.18	10	5	0.33	0.64
A31b	25	6	0.52	0.68	13	4	0.54	0.69	14	4	0.36	0.72	23	5	0.17	0.65	21	5	0.19	0.26
B11	29	7	0.65	0.70	30	4	0.41	0.56	37	2	0.03	0.13	42	5	0.17	0.20	21	7	0.57	0.79
B34	29	5	0.41	0.63	30	4	0.30	0.52	37	2	0.00	0.23	42	5	0.17	0.41	21	4	0.57	0.65
B59	29	12	0.55	0.87	30	7	0.20	0.82	37	8	0.11	0.67	42	7	0.05	0.64	21	8	0.57	0.81
C110	29	4	0.35	0.39	30	2	0.00	0.06	37	3	0.05	0.24	42	3	0.05	0.05	21	4	0.33	0.49
C133	29	6	0.79	0.74	30	4	0.13	0.13	7	4	0.03	0.13	42	3	0.02	0.05	21	4	0.81	0.67
C135b	29	7	0.48	0.68	30	2	0.32	0.43	37	4	0.05	0.27	42	4	0.12	0.16	21	6	0.47	0.77
C135c	13	10	0.31	0.83	10	6	0.27	0.74	10	2	0.00	0.24	19	4	0.05	0.25	19	9	0.68	0.79
C19	29	6	0.58	0.76	30	3	0.32	0.52	37	6	0.05	0.20	42	4	0.07	0.46	21	7	0.33	0.71
C32	11	5	0.27	0.56	13	6	0.46	0.68	8	2	0.13	0.12	9	2	0.11	0.45	9	4	0.22	0.50
C42	8	4	0.38	0.49	10	3	0.30	0.54	11	2	0.09	0.43	5	2	0.00	0.32	18	9	0.61	0.68
C49	29	4	0.59	0.61	30	2	0.23	0.45	37	5	0.08	0.15	42	3	0.05	0.05	21	8	0.52	0.73
C55	15	5	0.33	0.79	6	3	0.17	0.62	6	2	0.00	0.28	13	4	0.15	0.68	9	7	0.33	0.83
C67	29	5	0.43	0.46	30	5	0.40	0.61	37	3	0.03	0.08	42	5	0.08	0.38	21	5	0.62	0.72
C89	29	4	0.35	0.65	30	6	0.23	0.53	37	3	0.05	0.52	42	3	0.02	0.51	21	3	0.38	0.56
D17	9	4	0.50	0.65	10	7	0.40	0.87	10	1	0.00	0.00	11	2	0.36	0.46	11	7	0.54	0.75
E19b	23	12	0.70	0.86	15	3	0.47	0.52	21	6	0.10	0.67	19	2	0.00	0.15	12	7	0.42	0.73
E30	29	7	0.59	0.62	30	4	0.40	0.69	37	4	0.05	0.08	42	4	0.10	0.22	21	5	0.43	0.56
E42	29	6	0.34	0.36	30	2	0.00	0.06	37	2	0.08	0.21	42	3	0.00	0.18	21	6	0.62	0.75
E70	29	7	0.52	0.81	30	4	0.60	0.63	37	4	0.22	0.20	42	3	0.11	0.12	21	7	0.52	0.56
Mean[b]	23.29	6.24[A]	0.48[A]	0.66[A]	22.8	4.00[B]	0.31[B]	0.53[B]	26.0	3.48[B]	0.09[C]	0.29[C]	31.5	3.57[B]	0.09[C]	0.31[C]	18.2	6.05[A]	0.48[A]	0.66[A]

Note: A = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; LF-SC = large-flowered self-compatible; LF-SI = large-flowered self-incompatible; n = number of individuals screened/locus/population; SF-SC = small-flowered self-compatible.

[a] For an additional three loci, the number of individuals within each population was low and data were collected from >1 population within each mating type, and estimates calculated from the pooled sample of individuals within species. These data are provided here: A31c: *C. cheiranthifolia* n = 15, A = 8, H_o = 0.31, H_e = 0.78, *C. bistorta* n = 7, A = 4, H_o = 0.40, H_e = 0.76; C18: *C. cheiranthifolia* n = 23, A = 12, H_o = 0.26, H_e = 0.84, *C. bistorta* n = 8, A = 7, H_o = 0.5, H_e = 0.83; C66: *C. cheiranthifolia* n = 18, A = 9, H_o = 0.22, H_e = 0.88, *C. bistorta* n = 8, A = 8, H_o = 0.86, H_e = 0.86.

[b] Superscript capital letters beside mean values in each parameter measured represent significant ($P < 0.01$) differences between populations after paired one-tailed t test comparisons.

TABLE 3. Cross-amplification and allele sizes of 24 microsatellite primer pairs developed for *Camissoniopsis cheiranthifolia* and screened in *C. bistorta*, *C. micrantha*, *C. lewisii*, *Eulobus crassifolius*, *E. californicus*, *E. angelorum*, *Camissonia benitensis*, *C. strigulosa*, and *C. contorta*.[a,b]

Locus	*C. bistorta* (80, 12)	*C. micrantha* (14, 3)	*C. lewisii* (2, 1)	*E. crassifolius* (14, 3)	*E. angelorum* (6, 1)	*E. californicus* (5, 1)	*C. benitensis*[c] (213, 19)	*C. contorta*[c] (42, 2)	*C. strigulosa*[c] (62, 3)
A18	162–203	214	152–162	Failed	Failed	Failed	Failed	Failed	Failed
A31b	225–237	203–236	NT	NT	NT	NT	171–192[d] 180–202[d]	171–192[d] 180–202[d]	171–192[d] 180-202[d]
A31c	177–201	NT	NT	NT	NT	NT	NT	NT	NT
B11	122–148	145	133	131–143	131–135	131–135	Failed	Failed	Failed
B34	239–251	247–249	249	245–255	245–249	Failed	183–236	183–236	183–236
B59	123–157	121–143	127–147	121–167	Failed	Failed	NT	NT	NT
C110	192–204	192–202	202	188–202	194–204	194–202	Failed	Failed	Failed
C133	121–149	142–158	142	142–149	141–145	142–148	Failed	Failed	Failed
C135b	131–177	139–143	142	Failed	Failed	Failed	Failed	Failed	Failed
C135c	218–274	NT	NT	NT	NT	NT	NT	NT	NT
C18	177–201	NT	NT	NT	NT	NT	NT	NT	NT
C19	221–255	226–236	232	221–247	Failed	Failed	Failed	Failed	Failed
C32	187–209	216	221	172–216	Failed	Failed	Failed	Failed	Failed
C42	235–273	235–245	Failed	Failed	206–246	235–245	166–174	166–174	166–174
C49	191–231	198–202	196	196–208	196–208	195–208	Failed	Failed	Failed
C55	235–273	NT	NT	NT	NT	NT	NT	NT	NT
C66	209–247	NT	NT	NT	NT	NT	NT	NT	NT
C67	233–257	237–249	173–241	Failed	Failed	235–245	209–219	209–219	209–219
C89	194–222	200	202	165–204[d] 314–332[d]	298–326	314–334	NT	NT	NT
E19b	205–247	NT	NT	NT	NT	NT	NT	NT	NT
E30	179–214	188–198	192–194	192–282	181–247	213–235	177–187	177–187	177–187
E42	179–202	180–192	191	180–188	Failed	Failed	Failed	Failed	Failed
E70	124–144	126–136	133–145	122–144	129–139	133–143	103–119	103–119	103–119
D17	219–249	NT	Failed	Failed	Failed	Failed	Failed	Failed	Failed

[a] Total numbers of individuals from the populations sampled are indicated in parentheses.

[b] Amplification failures (Failed) and loci that were not tested in some species (NT) are indicated.

[c] Data for the three species of *Camissonia* are from Dick et al. (2014).

[d] These primers amplified two non-overlapping variable regions in the species indicated, so two fragment ranges are provided.

closely related taxa, providing opportunities to test a broad range of ecological and evolutionary questions within species and across taxa. These markers will facilitate our ongoing studies of mating system evolution and geographic range limits in *C. cheiranthifolia*, as well as the genetic and ecological consequences of hybridization between *C. cheiranthifolia* and *C. bistorta*. The high frequency of cross-amplification in related taxa provides opportunities for comparative studies investigating the genetic consequences of variation in life history and mating system, and ongoing hybridization in this morphologically and ecologically variable group.

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Appendix 1. Location and sampling information, population codes, and mating type of individuals used in this study.

Location	Population code	Taxa sampled[a]	Latitude (°N)	Longitude (°W)	*n*	Mating and floral type[b]	Herbarium accession no.[c]
Mexico, Baja California							
Guerrero Negro	BGN	cr	27.9556	−114.0670	5	LF-SI	SD92680
Transpeninsular Hwy. near Santa Ana	BTH	an	29.09152	−114.15297	6	LF-SI	SD144733
Bocana del Rosario	BBR	ch	30.0478	−115.7863	7	SF-SC	SD95717
Bocana del Rosario	BBR	cr	30.1691	−115.7973	5	LF-SI	SD91289
El Socorro	BES	ch	30.3186	−115.8257	37	SF-SC	SD52704
El Socorro	BES	le	30.3235	−115.8186	2	SF-SC	SD11800
Bahia Santa Maria	BBS	cr	30.3973	−115.9051	4	LF-SI	UCR41467
Bahia San Quinín	BBQ	ch	30.3801	−115.9904	5	SF-SC	UCR38448
Bahia Falsa	BBF	ch	30.4558	−116.0342	5	SF-SC	SD91177
La Chorera	BCH	ca	30.4782	−115.9929	5	LF-SI	ASU0033348
San Martin Island	BSM	ch	30.48312	−116.1022	6	SF-SC	SD77648
Ejido Leandro Valle, northwest of San Quinín	BQW	ch	30.7058	−116.0356	4	SF-SC	SD91177
San Antonio del Mar	BSA	ch	31.1077	−116.3084	5	SF-SC	SD124971
Punta Banda sand spit	BPB	bi	31.7258	−116.6481	6	LF-SI	SD93875
Ensenada beaches	BEB	ch	31.8102	−116.6092	5	LF-SI	SD64735
La Mission, scenic Hwy.	BMI	bi	32.0946	−116.8811	5	LF-SI	SD72998
Los Arenales	BLA	bi	32.2067	−116.9147	5	LF-SI	SDSU3341
Paseo playas de Tijuana	BTB	bi	32.5202	−117.1229	6	LF-SI	SD101764
USA, California							
Borderfields SP bluffs	CBF	bi	32.5355	−117.1189	5	LF-SI	SD181102
Borderfields SP sand dunes	CBF	ch	32.5365	−117.1229	29	LF-SI	SD83479
Silver Strand	CSS	mi	32.6385	−117.1425	5	LF-SI	SD189780
Silver Strand	CSS	ch	32.6410	−117.1437	6	LF-SI	SD38644
Willow Glen Dr.	CWG	bi	32.7568	−116.9011	6	LF-SI	SD176653
Cuyamaca Street	CCU	bi	32.84763	−116.98145	21	LF-SI	SDSU3338
El Monte	CEM	bi	32.8926	−116.8470	5	LF-SI	SD3324
Torry Pines SP	CTP	bi	32.9187	−117.2584	5	LF-SI	SD181105
Torry Pines SP	CTP	ch	32.9290	−117.2591	6	LF-SI	SD227356
Camp Pendleton	CCP	ch	33.2484	−117.4300	5	LF-SI	SD203540
San Nicolas Island (big dune)	CSN3	ch	33.2655	−119.4972	4	SF-SC	SD70471
San Nicolas Island (naval facility)	CSN2	ch	33.2572	−119.5617	3	LF-SC	SBBG117416
San Nicolas Island (canyon)	CSN1	ch	33.2707	−119.5434	3	SF-SC	SBBG33797
San Onofre SP	CSO	ch	33.3808	−117.5770	5	LF-SI	DS510009
San Onofre SP	CSO	bi	33.3964	−117.5898	5	LF-SC	SD124489
Dana Point Preserve	CDP	ch	33.4607	−117.7155	5	LF-SI	UCR203990
Dana Point Preserve	CDP	bi	33.46247	−117.7133	5	LF-SI	UCR215311
Dockweiler SB	CDW	ch	33.9235	−118.4320	4	LF-SC	SD38668
Santa Rosa—China Camp	CSR2	ch	33.9293	−120.1782	3	SF-SC	SBBG36622
Santa Rosa—Skunk Point	CSR1	ch	33.9798	−119.9973	4	SF-SC	POM171247
Santa Cruz—Sauce Beach	CSC2	ch	34.0108	−119.8829	5	SF-SC	SD229734
Santa Rosa—Carrington Point	CSR3	ch	34.0241	−120.0700	5	SF-SC	RSA132262
Santa Cruz—Fraser Point	CSC1	ch	34.0571	−119.9220	4	SF-SC	SBBG53934
Ormond Beach	COR	ch	34.1399	−119.1893	4	LF-SC	UC57062
Point Mugu SP	*CPM	ch	34.11447	−119.1494	4	LF-SC	SBBG95027
McGrath SB	CMG	ch	34.2246	−119.2592	6	LF-SC	SBBG14459
San Buenaventura SB	CBV	ch	34.2679	−119.2783	5	LF-SC	RSA44553
Santa Paula	CSP	bi	34.3558	−119.0369	6	LF-SI	SBBG124315
Coal Oil Point	*CCO	ch	34.4083	−119.8793	30	LF-SC	SD38666
Guadalupe Nipomo	CGN3	ch	34.9504	−120.6535	7	SF-SC	CAS297044
Guadalupe Nipomo	CGN2	mi	35.0258	−120.6331	5	SF-SC	SD38675
Guadalupe Nipomo	CGN2	ch	35.0287	−120.6323	6	SF-SC	SDSU19557
Morro Bay Strand	CMS	ch	35.3986	−120.8669	6	SF-SC	CAS690774
Point Lobos SP	CPL	ch	36.5171	−121.9512	5	SF-SC	CAS323912
Salinas River	CSA	ch	36.7745	−121.7956	5	SF-SC	UCD103530
Sun Set Beach SP	CST	ch	36.8766	−121.8252	5	SF-SC	UC942887
Sun Set Beach SP	CST	mi	36.8782	−121.8262	4	SF-SC	RSA187219
Wilder Ranch	CWR	ch	36.9541	−122.0799	5	SF-SC	POM38414
Point Reyes NP	CPR2	ch	38.0461	−122.9879	7	SF-SC	RSA119359
Manchester Beach SP	CMC	ch	38.9827	−123.7057	42	SF-SC	CAS807342
Manilla Dunes Community Center	CMA	ch	40.8474	−124.1738	6	SF-SC	HSC45467
Tolowa Dunes SP	CTD	ch	41.8705	−124.1738	5	SF-SC	POM305910

Appendix 1. Continued.

Location	Population code	Taxa sampled[a]	Latitude (°N)	Longitude (°W)	*n*	Mating and floral type[b]	Herbarium accession no.[c]
USA, Oregon							
Pistol River	OPR	ch	42.2709	−124.4049	5	SF-SC	OSC62832
Bullards Beach SP	OBU	ch	43.1463	−124.4151	4	SF-SC	CM485480
North Spit Overlook	ONO	ch	42.2709	−124.4049	7	SF-SC	WS316639

Note: *n* = number of individuals assayed; NP = National Park; SB = State beach; SP = State park.

[a] Species: *Camissoniopsis cheiranthifolia* (ch), *Camissoniopsis bistorta* (bi), *Camissoniopsis micrantha* (mi), *Camissoniopsis lewisii* (le), *Eulobus angelorum* (an), *Eulobus crassifolius* (cr), *Eulobus californicus* (ca).

[b] Mating types: LF-SC = large-flowered self-compatible, LF-SI = large-flowered self-incompatible, SF-SC = small-flowered self-compatible.

[c] Herbarium accession numbers from specimens collected at each of the sampling locations or nearby locations are provided for each population sampled. Herbaria codes: ASU = Arizona State University, Tempe; CAS or DS = California Academy of Sciences, San Francisco; CM = Carnegie Museum of Natural History; HSC = Humboldt State University Herbarium; OSC = Oregon State University; POM and RSA = Rancho Santa Ana Botanic Garden; SBBG = Santa Barbara Botanic Garden Herbarium; SD = San Diego Natural History Museum; SDSU = San Diego State University, San Diego; UC = University of California, Berkeley; UCD = University of California, Davis; UCR = University of California, Riverside; WS = Washington State University.

* One plant from each of these two populations was used for the construction of the genomic library.