MICROSATELLITE PRIMERS FOR CAMISSONIOPSIS CHEIRANTHIFOLIA (ONAGRACEAE) AND CROSS-AMPLIFICATION IN RELATED SPECIES

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In the northern range limit in southern Oregon are extensive populations in Baja California toward the southern range limit, on 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). 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 largely self-incompatible (SI), 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 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.

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 AluI + HaeIII + Rad restriction enzymes. Digested DNA was desphosphorylated using 0.01 unit calf intestinal alkaline phosphatase per micromole 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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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% Tris [pH 8.0], 1 mM EDTA). DNA quality and size were evaluated on 1.5% agarose gels (fragments ranged from 200–1000 bp). DNA was extracted from 115 positive clones with appropriate insert sizes were estimated with PCR using M13 primers and verified on 1% agarose gels. DNA extraction was performed 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; 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).

For two loci (A31b and C135b), we developed two additional primer pairs (A31c and C135c; see text for details). Note: T_a = annealing temperature.

| Locus   | Primer sequences (5'-3') | Repeat motif | T_a (°C) | Allele size range (bp) | Multiplexed | GenBank accession no. |
|---------|-------------------------|--------------|----------|------------------------|-------------|----------------------|
| A18     | F: TCTCGTGTGTTGCCTTTCTT | (TC)_28      | 55       | 212–225                | single      | Pr032165043          |
| A18     | R: CCTGACAGAAAGACATGG   | (TC)_10      | 55       | 220–242                | single      | Pr032165044          |
| B11     | F: CCGAAGAAATGAAAGTTGCC | (GA)_9       | 55       | 120–152                | 2plex1      | Pr032165046          |
| B34     | F: TTTACCTCGCTTATATTTGT | (TC)_10      | 57       | 237–253                | 2plex2      | Pr032165047          |
| B59     | F: CCTACACACTGGCAGCTGT  | (TC)_25      | 57       | 121–179                | 2plex5      | Pr032165048          |
| C110    | F: AACGACGAGGAACACGAG   | (GA)_9       | 57       | 194–210                | 2plex2      | Pr032165049          |
| C133    | F: GGCCTGCTAGGAGAAGAT   | (GA)_14      | 55       | 121–157                | 2plex4      | Pr032165050          |
| C135b   | F: ACAGTGAGTGTTTCAATTC  | (TC)_12      | 57       | 131–149                | 3plex       | Pr032165051          |
| C135c   | F: CGCGCTTACATCGTACTCA  | (TC)_12      | 57       | 219–255                | single      | Pr032165052          |
| C18     | F: CCTGGCTGACTTCTCATTGT | (GA)_15      | 57       | 173–211                | single      | Pr032165053          |
| C19     | F: GCCCTCTCTTATGCAATGCT | (GA)_14      | 57       | 222–316                | 3plex       | Pr032165054          |
| C32     | F: TCTCTCTCTCCTCTCCTCT  | (GA)_14      | 55       | 189–217                | single      | Pr032165055          |
| C42     | F: CCTGAAATCAGTCTGATA   | (TA)_15      | 57       | 243–255                | single      | Pr032165056          |
| C49     | F: GCAGGCAATAGGTTTACA   | (TG)_12      | 57       | 196–214                | 3plex       | Pr032165057          |
| C55     | F: AAAGGAGAGACACGGCTTT  | (GA)_14      | 57       | 123–155                | single      | Pr032165058          |
| C66     | F: TGCTTATAGGATGATAGGCT | (GA)_3      | 57       | 209–247                | single      | Pr032165059          |
| C67     | F: GAAGACGAGATCAAGACAG  | (TC)_15      | 57       | 233–257                | 2plex3      | Pr032165060          |
| C89     | F: TGAATACATCGACCGGACTA | (TA)_3(GA)_6 | 57       | 196–212                | 2plex5      | Pr032165061          |
| C10     | F: CACAGCTGACAGTTCGCT   | (TC)_12      | 57       | 219–255                | single      | Pr032165052          |
| E19b    | F: CTTTCACAAATATGGAAGA  | (TA)_2(GA)_6 | 57       | 215–249                | single      | Pr032165062          |
| E30     | F: CATTCAGTGGTCAGTGGAT  | (TC)_12      | 55       | 205–247                | single      | Pr032165063          |
| E42     | F: TGTCCTCCTCTGCTGAGTG  | (GA)_10      | 55       | 179–197                | 2plex4      | Pr032165065          |
| E70     | F: GATAGTCTGCTCACAATGCA  | (TC)_15      | 57       | 128–144                | 2plex3      | Pr032165066          |

Note: T_a = annealing temperature.

*Range of fragment sizes including the M13 tag (5'-CACGACGTTGTAACACGAG-3') attached to the forward primer.

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).

For two loci (A31b and C135b), we developed two additional primer pairs (A31c and C135c; see text for details).
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 totally, of which 56 were found only in C. cheiranthifolia (mean ± 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. Hs and Ht 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. lewissii (P.H. Raven) W.L. Wagner & Hoch, Eulobus angelerum (S. Watson) W.L. Wagner & Hoch, E. californicus Nutt. ex Torr. & A. Gray, and C. crassifolia (Greene) W.L. Wagner & Hoch, and successful amplification occurred for 17, 15, 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 congers C. rigida (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
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 *Camissonia cheiranthifolia*, as well as the genetic and ecological consequences of hybridization between *Camissonia 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 | Latitude (°N) | Longitude (°W) | n | Mating and floral type | Herbarium accession no. |
|----------|-----------------|--------------|---------------|----------------|----|-----------------------|------------------------|
| **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 |
| El Socorro | BES | ch | 30.3186 | −115.8257 | 37 | SF-SC | SD52704 |
| Bahia Santa Maria | BBS | cr | 30.3973 | −115.9051 | 4 | LF-SI | UCR41467 |
| Bahia San Quín | BBQ | ch | 30.3801 | −115.9904 | 5 | SF-SC | UCR38448 |
| Bahia False | 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 Quín | BQW | ch | 30.7058 | −116.0356 | 4 | SF-SC | SD91177 |
| **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 | SF-SC | SD189780 |
| Silver Strand | CSS | ch | 32.6410 | −117.1437 | 6 | SF-SC | SD83644 |
| Willow Glen Dr. | CWW | bi | 32.7568 | −116.9011 | 6 | SF-SC | SD176653 |
| Cuyamaca Street | CUC | ch | 32.84763 | −116.98145 | 21 | LF-SI | SD133338 |
| El Monte | CEM | ch | 32.8926 | −116.8470 | 5 | SF-SC | SD3324 |
| Torry Pines SP | CTP | bi | 32.9187 | −117.2584 | 5 | SF-SC | SD181105 |
| Torry Pines SP | CTP | ch | 32.9290 | −117.2591 | 6 | SF-SC | SD227356 |
| Camp Pendleton | CCP | ch | 33.2484 | −117.4300 | 5 | SF-SC | SD20534 |
| 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 | DS50009 |
| San Onofre SP | CSO | bi | 33.3964 | −117.5898 | 5 | SF-SC | SD124489 |
| Dana Point Preserve | CDP | ch | 33.4607 | −117.7155 | 5 | LF-SI | UCR203990 |
| Dana Point Preserve | CDP | ch | 33.46247 | −117.7133 | 5 | SF-SC | 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 | CSR3 | ch | 34.0241 | −120.0700 | 5 | SF-SC | RSA132626 |
| Point Santa Cruz—Fraser Point | CSC1 | ch | 34.0571 | −119.9220 | 4 | SF-SC | SBBG53934 |
| Ormond Beach | COR | ch | 34.1399 | −119.1893 | 4 | SF-SC | UC57062 |
| Point Mugu SP | *CPM | ch | 34.11447 | −119.1494 | 4 | SF-SC | SBBG95027 |
| McGrath SB | CMG | ch | 34.2246 | −119.2592 | 6 | SF-SC | SBBG14459 |
| San Buenaventura SB | CBB | ch | 34.2679 | −119.2783 | 5 | SF-SC | RSA44553 |
| Santa Paula | CSP | bi | 34.3558 | −119.0360 | 6 | LF-SI | SBBG124315 |
| Coal Oil Point | *CCO | ch | 34.4083 | −119.8973 | 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 | SD83675 |
| Guadalupe Nipomo | CGN2 | ch | 35.0287 | −120.6323 | 6 | SF-SC | SD5019557 |
| Morro Bay Strand | CMS | ch | 35.3986 | −120.8666 | 6 | SF-SC | CAS690774 |
| Point Lobos SP | CPL | ch | 36.5171 | −121.9512 | 5 | SF-SC | CAS3233912 |
| 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 | UCR42887 |
| 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 |

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APPENDIX 1. Continued.

| Location               | Population code | Taxa sampled | Latitude (°N) | Longitude (°W) | n  | Mating and floral type | Herbarium accession no. |
|------------------------|-----------------|--------------|---------------|----------------|----|------------------------|------------------------|
| 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.
Species: *Camissoniopsis cheiranthifolia* (ch), *Camissoniopsis bistorta* (bi), *Camissoniopsis micrantha* (mi), *Camissoniopsis lewisii* (le), *Eulobus angelorum* (an), *Eulobus crassifolius* (cr), *Eulobus californicus* (ca).
Mating types: LF-SC = large-flowered self-compatible, LF-SI = large-flowered self-incompatible, SF-SC = small-flowered self-compatible.
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.