Development of 15 microsatellite loci in the endangered *Streptanthus glandulosus* subsp. *niger* (Brassicaceae)

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**PREMISE OF THE STUDY:** The endangered *Streptanthus glandulosus* subsp. *niger* (Brassicaceae) is endemic to a single peninsula in California and threatened by fragmentation. We developed microsatellite markers to investigate genetic diversity in the two extant populations and the degree to which they have diverged from one another.

**METHODS AND RESULTS:** We used Illumina HiSeq high-throughput sequencing to develop 15 microsatellite markers, 14 of which were polymorphic. These di- and trinucleotide repeats yielded one to 11 alleles per locus in 61 plants across the two populations. Levels of observed and expected heterozygosities ranged from 0.108 to 0.946 and 0.257 to 0.839, respectively. We demonstrated cross-amplification in a second rare subspecies, *S. glandulosus* subsp. *secundus*, and in the widespread congener *S. tortuosus*.

**CONCLUSIONS:** These are the first microsatellites reported for this subspecies, and they will aid in the inclusion of genetic information in conservation planning. Cross-amplification was demonstrated in two related taxa, including one of conservation concern.

**KEY WORDS** Brassicaceae; conservation; fragmentation; microsatellite; population genetics; *Streptanthus glandulosus* subsp. *niger*; *Streptanthus glandulosus* subsp. *secundus*; *Streptanthus tortuosus*.

The genus *Streptanthus* Nutt. (Brassicaceae) is noteworthy for the remarkable morphological diversity, adaptations to unusual soil types, and rarity of its ~35 species (Cacho et al., 2014). *Streptanthus glandulosus* Hook. subsp. *niger* (Greene) Al-Shehbaz, M. S. Mayer & D. W. Taylor is adapted to serpentine soils that are characterized by high concentrations of heavy metals and low concentrations of macronutrients. It has one of the most restricted ranges of all members of this group, confined to the 6-km-long Tiburon Peninsula (Marin County, California, USA). It is pollinated by bees and is capable of self-pollination.

*Streptanthus glandulosus* subsp. *niger* occupied a large portion of the Tiburon Peninsula prior to suburban development in the mid-20th century but now persists as two populations separated by 1.5 km of dense housing and is listed as Endangered at both the state and federal levels (California Department of Fish and Wildlife, 2014; U.S. Fish and Wildlife Service, 2019). The population at Middle Ridge Park is smaller than the population at Old St. Hilary’s Preserve. The total number of plants ranges from ~500 to 3000 depending on annual precipitation (Swope, unpublished data). Nothing is known about the genetic diversity or structure of the two remaining populations or gene flow between them. Although microsatellites are widely used to answer these questions and may be useful in conservation planning, none exist for this subspecies.

**METHODS AND RESULTS**

Fresh leaves were collected from 40 individuals at Old St. Hilary’s Preserve and 24 at Middle Ridge Park, stored in separate envelopes at room temperature with silica beads (PolyLam Products, Williamsville, New York, USA), and extracted within one week of collection. DNA was extracted by placing 3–4 mm of dried leaf tissue in 300 μL of a 10% Chelex solution (Bio-Rad Laboratories, Hercules, California, USA), vortexing samples for 10 s, then spinning them for another 10 s to ensure that plant material was in the solution. The solution was incubated at 65°C for 10 min, followed by another round of vortexing for 10 s. Finally, we centrifuged the samples for 10 s to separate contaminants and Chelex beads from the DNA in the supernatant. We diluted the supernatant from the Chelex extraction 1 : 1 with distilled H₂O.

A microsatellite library was created using extracted genomic DNA from three individuals, one from the Middle Ridge Park population and two collected from opposite ends of the larger Old St.
Hilary’s Preserve population. Due to restrictions on collecting, our specimens were vouched with existing herbarium collections (Appendix 1). DNA was sent to the QB3 Genomics Sequencing Laboratory at the University of California, Berkeley, for shotgun library preparation and 2 × 300-bp paired-end Illumina HiSeq genome sequencing. Approximately nine million FASTQ reads per sample were filtered for minimum length (50 bp) and trimmed for quality using Trimmomatic version 0.23.3 (Bolger et al., 2014) using a window size of 4 bp with a threshold average quality of Phred = 28 sister taxon to S. glandulosus 2 of 4

### TABLE 1. Characteristics of 15 microsatellite loci developed for Streptanthus glandulosus subsp. niger.

| Locus | Primer sequences (5 ’→3’) | Repeat motif | Allele size range (bp) | Tm (°C) | Fluorescent label | GenBank accession no. |
|-------|---------------------------|--------------|------------------------|---------|-------------------|----------------------|
| Sn50  | F: CTATGTCTTCTCATAAACCTCACAAGCC | (CAA)4 | 132–138 | 68 | FAM | MG029359 |
| Sn255 | F: GGTCCAGAAGAAGAAGCTCAGC | (CT)10 | 138–182 | 66 | FAM | MG029360 |
| Sn262 | F: CATCCTAATCCCTGTGAGATGAAAACG | (CT)4 | 147–175 | 64 | FAM | MG029357 |
| Sn313 | F: GAATTTGACCTGGAGATGAAAACG | (AT)10 | 157–171 | 65 | HEX | MH316554 |
| Sn347 | F: CAAATGTCCTTGACATCTATACACC | (TA)4 | 95–105 | 63 | FAM | MG029355 |
| Sn430 | F: GGGAGGTGTTACATGCTTAAGGGG | (TA)4 | 131–145 | 67 | FAM | MG029364 |
| Sn463 | F: AGAGAGGTTGATGCTGTTGAAGGC | (AAC)4 | 82–109 | 67 | FAM | MG029358 |
| Sn558 | F: GGAGATGACTGAGACAGATCCTCACAG | (TC)4 | 92–114 | 64 | HEX | MG029356 |
| Sn588 | F: GAACTGTTGGCCTACCTCCTGCG | (TCC)4 | 128–182 | 63 | HEX | MG029361 |
| Sn715 | F: CCCGTCATTCTCTACAAGCTAGTCG | (TA)4 | 92–118 | 68 | FAM | MH316555 |
| Sn803 | F: GTTTAATGTGCTTGAAGGAGCTG | (TA)4 | 141–197 | 63 | FAM | MG029362 |
| Sn1015 | F: TCATGTAATACGGGAGAGAGTECCA | (TA)4 | 96–154 | 66 | HEX | MH316558 |
| Sn1434 | F: CACCGAATCTGGTACTGTTGG | (TA)4 | 81 | 64 | HEX | MH316552 |
| Sn1618 | F: GAAATAAGAGAGAGATGCTCTTTCG | (CTT)4 | 139–169 | 65 | FAM | MG029363 |
| Sn2378 | F: CAACAGTGTATATCAATTTGATATCACTGG | (TAA)4 | 124–220 | 64 | FAM | MH316557 |

Note: Tm = annealing temperature.

**Characteristics of 15 microsatellite loci developed for Streptanthus glandulosus subsp. niger.**

Primers for the 41 loci were designed to have 35–55% GC content, a target melting temperature of 64–65°C (salt-adjusted, 50 mM NaCl), and an amplicon size of 90–150 bp. A subset of 23 loci were screened for amplification by electrophoresing the products on a 4% SFR agarose gel (VWR Life Science, Philadelphia, Pennsylvania, USA) with an Invitrogen 100-bp ladder (Invitrogen, Carlsbad, California, USA) using nine S. glandulosus subsp. niger individuals. Of those 23 loci, 15 produced consistent and robust amplification products and thus were suitable for genotyping. Sequence library data were deposited into the National Center for Biotechnology Information Sequence Read Archive (BioProject ID PRJNA503999).

PCR conditions were optimized using a Bio-Rad T100 Thermal Cycler (Bio-Rad Laboratories). Amplification reactions were universally conserved across the tribe (Warwick and Al-Shehbaz, 2006). Rare reports of unusual karyotypes (e.g., 2n = 14, 2n = 56) show no phylogenetic pattern (Cacho et al., 2014) and thus appear to be sporadic in nature. In Caulanthus amplexicaulis S. Watson, a 2n = 28 sister taxon to S. glandulosus subsp. niger, 250 of 258 microsatellite markers (97%) produced a single amplification product from each of two distinct homoyzogous inbred lines. Considered together, these lines of evidence justify the selection of diploid as a ploidy parameter. Genomic coordinates identified as microsatellites by misa.pl were thus filtered for the presence of dimeric and trimeric repeats in all three assemblies that were in all cases called by NGSEP as “STR” and “INDEL” with a minimum confidence score of 100. Forty-one microsatellite loci met these criteria.

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singleplexed in a final volume of 25.5 μL containing approximately 2 ng of DNA, 12.5 μL of Q5 High-Fidelity DNA Polymerase (New England BioLabs, Ipswich, Massachusetts, USA), 1 μL of Milli-Q water (MilliporeSigma, Burlington, Massachusetts, USA), and 0.5 μM of each forward and reverse primer. The PCR program consisted of one cycle of denaturation at 98°C for 30 s; followed by 34 cycles at 98°C for 40 s, 60°C for 30 s, 72°C for 20 s; and an extension phase at 72°C for 1 min. Forward primers were fluorescently labeled with HEX or FAM (Eurofins, Louisville, Kentucky, USA) and tested for cross-amplification in six and two congeners. Optimization of these markers will be useful in quantifying the genetic diversity in the two remaining populations of S. glandulosus subsp. niger and the degree to which these populations have diverged from one another. These markers may also be useful in comparative studies with S. glandulosus subsp. secundus (Table 2).

CONCLUSIONS

We developed microsatellite markers for the endangered S. glandulosus subsp. niger and two congeners. Optimization of these markers will be useful in quantifying the genetic diversity in the two remaining populations of S. glandulosus subsp. niger and the degree to which these populations have diverged from one another. These markers may also be useful in comparative studies with S. glandulosus subsp. secundus, which is also of conservation concern.

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AUTHOR CONTRIBUTIONS

S.M.S. conceived of the project, collected leaf tissue, supervised the lab and field work, conducted allele scoring and analyses, and wrote nearly the entire 1200-km length of California. Eleven loci successfully amplified in both taxa (Table 2), producing one or two resolvable amplification products per individual, suggesting they amplified a single locus. Three loci produced no amplification products in either species, and one locus amplified more than one product in S. glandulosus subsp. secundus (Table 2).
the manuscript. A.E.P. conducted the whole-genome shotgun sequencing, contig assembly, and variant calling, and co-authored the manuscript. G.T.L., B.A.B., and H.M.H. conducted the lab work and reviewed the manuscript.

**DATA ACCESSIBILITY**

Sequence library data were deposited in the National Center for Biotechnology Information (NCBI) Short Read Archive (BioProject ID PRJNA503999). Sequence information for the developed primers has been deposited to NCBI; GenBank accession numbers are provided in Table 1.

**LITERATURE CITED**

Beier, S., T. Thiel, T. Münch, U. Scholz, and M. Mascher. 2017. MISA-web: A web server for microsatellite prediction. *Bioinformatics* 33: 2583–2585.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30: 2114–2120.

Cacho, N. I., A. M. Burrell, A. E. Pepper, and S. Y. Strauss. 2014. Novel nuclear markers inform the systematics and the evolution of serpentine use in *Streptanthus* and allies (*Thelypodieae, Brassicaceae*). *Molecular Phylogenetics and Evolution* 72: 71–81.

California Department of Fish and Wildlife. 2014. California Threatened and Endangered plant profiles: Tiburon jewelweed (*Streptanthus glandulosus ssp. niger*). Website https://www.wildlife.ca.gov/Conservation/Plants/Endangered/Streptanthus-glandulosus-ssp-niger [accessed 8 January 2019].

Hawkins, A. K., E. R. Garza, V. A. Dietz, O. J. Hernandez, W. D. Hawkins, A. M. Burrell, and A. E. Pepper. 2017. Transcriptome signatures of selection, drift, introgression, and gene duplication in the evolution of an extremophile endemic plant. *Genome Biology and Evolution* 9: 3478–3494.

Kagale, S., S. J. Robinson, J. Nixon, R. Xiao, T. Huebert, J. Condie, D. Kessler, et al. 2014. Polyploid evolution of the Brassicaceae during the Cenozoic era. *Plant Cell* 26: 2777–2791.

Langmead, B., and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. *Nature Methods* 9: 357–359.

Peakall, R. O. D., and P. E. Smouse. 2012. GenEALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* 28: 2537–2539.

Perea, C., J. F. De La Hoz, D. F. Cruz, J. D. Lobaton, P. Izquierdo, J. C. Quintero, B. Raatz, and J. Duitama. 2016. Bioinformatic analysis of genotype by sequencing (GBS) data with NGSEP. *BMC Genomics* 17(Supplement 5): 498.

Rousset, F. 2008. GENEPOP: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106.

Simpson, J. T. K., W. D. Hawkins, R. D. Williams, and P. Shipley. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535–538.

Warwick, S. I., and I. A. Al-Shehbaz. 2006. Brassicaceae: Chromosome number index and database on CD-Rom. *Plant Systematics and Evolution* 259: 237–248.

**APPENDIX 1.** Voucher and location information for species used in the development and evaluation of microsatellite markers for *Streptanthus glandulosus ssp. niger.*

| Taxon | Collection locality | Geographic coordinates | Voucher specimen accession no. |
|-------|---------------------|------------------------|-------------------------------|
| *Streptanthus glandulosus* Hook. ssp. *niger* (Greene) Al-Shehbaz, M. S. Mayer & D. W. Taylor | Tiburon Peninsula, Marin Co., CA, USA | 37°52′N, 122°27′W | CAS-BOT-BC87245, CAS-BOT-BC87246, CAS-BOT-BC87253, UC163931, JEPS2879, JEPS3882, JEPS77161, JEPS9284 |
| *Streptanthus glandulosus* ssp. *secundus* Greene | Carson Ridge, Marin Co., CA, USA | 37°57′N, 122°37′W | CAS-BOT-BC87281, UC1492744 |
| *Streptanthus tortuosus* Kellogg | Bolinas Road, Marin Co., CA, USA | 37°57′N, 122°37′W | CAS-BOT-BC87272 |
| | Terra Linda-Sleepy Hollow, Marin Co., CA, USA | 38°01′N, 122°34′W | CAS-BOT-BC87286 |
| | Gold Lake region, Plumas Co., CA, USA | 39°56′36″N, 121°08′03″W | CAS-BOT-BC87795 |
| | Confluence of Rock Creek and north fork of Feather River, Plumas Co., CA, USA | 39°54′01″N, 121°21′31″W | CAS-BOT-BC87797 |
| | Confluence of Granite Creek and north fork of Feather River, Plumas Co., CA, USA | 39°57′20″N, 121°17′44″W | CAS-BOT-BC87783 |
| | East fork of Feather River, north of Red Hill, Plumas Co., CA, USA | 40°03′21″N, 121°12′28″W | CAS-BOT-BC87785 |

*Due to the protected status of *S. glandulosus* ssp. *niger*, new voucher specimens were not collected for this study; the vouchered specimens listed here are representative of the plants used in this study.

*Geographic coordinates for *S. glandulosus* ssp. *niger* and *S. glandulosus* ssp. *secundus* have been reduced to minutes due to the protected status of these taxa.

*Vouchers with the prefix CAS are deposited at the California Academy of Sciences Herbarium (CAS), San Francisco, California, USA; vouchers with the prefixes UC and JEPS are deposited at University and Jepson Herbaria (JEPS), University of California, Berkeley, California, USA.*