MICROSATELLITE LOCI DEVELOPMENT IN MYCOTHEROTROPHIC
CORALLORHIZA MACULATA (ORCHIDACEAE) WITH
AMPLIFICATION IN C. MERTENSIANA

SARAH E. HOPKINS²,⁴ AND D. LEE TAYLOR³

¹Department of Biology and Wildlife, University of Alaska Fairbanks, 211 Irving I building, Fairbanks, Alaska 99775 USA; and ²Institute of Arctic Biology, University of Alaska Fairbanks, 311 Irving I building, Fairbanks, Alaska 99775 USA

• Premise of the study: Microsatellite primers were developed for the first time in the species Corallorhiza maculata, a nonphotosynthetic orchid that is becoming a model for studying mycorrhizal specificity.
• Methods and Results: Eight polymorphic microsatellite markers were developed using an enrichment and cloning protocol. The number of alleles for each locus ranged from two to seven. The loci were tested in three populations, and the resulting high $F_{ST}$ values suggested that these loci have utility in illuminating population structure. Cross-amplification was also achieved in the sister species C. mertensiana.
• Conclusions: These microsatellite markers will be useful in further study of the population genetics of this species, including that of previously described mycorrhizal races.

Key words: Corallorhiza maculata; cross-species amplification; microsatellite; orchids.

Corallorhiza maculata (Raf.) Raf. is a nonphotosynthetic terrestrial orchid that is becoming a model for studies of mycorrhizal specificity. It typically grows in late succession forests of pine, fir, or oak and is distributed throughout Mexico and the entire United States with the exception of the Great Plains region and the Southeast (Freudenstein, 1997). Because the plant is achlorophyllous, all of its carbon is acquired via a symbiotic relationship with a fungus in which the orchid appears to be acting parasitically. Like many parasite–host relationships, the group of fungi that the orchid exploits is narrow (Taylor and Bruns, 1999). Using three single nucleotide polymorphisms, Taylor et al. (2004) determined that there are at least six distinct genotypes or races of C. maculata, each of which associates with a different clade of ectomycorrhizal fungi in the family Russulaceae. Despite this fine-scale specialization, however, different genotypes can be found growing intermixed at a single site with each genotype utilizing a different fungus. Because prior genotyping was coarse, there is a need for higher-resolution genetic markers. Microsatellite markers should enable improved understandings of the patterns of gene flow in relation to mycorrhizal specificity within this species. Because C. maculata is a member of a species complex consisting of C. mertensiana Bong., C. bulbosa A. Rich. & Galeotti, and C. williamsii Correll, the microsatellite markers may also be useful for several other species. Here we report the development and application of eight microsatellite loci. The loci were developed for C. maculata and tested on three populations (Coastal California, Interior Northern California, and the Rocky Mountains in Colorado). The loci were also tested on a population of C. mertensiana, which is the only other widespread species within the C. maculata complex.

METHODS AND RESULTS

Floral and rhizome tissues were used as sources for genomic DNA of five orchid species (including C. maculata) by extraction using the Plant DNeasy Mini kit (QIAGEN, Valencia, California, USA). DNA of the five species were gel quantified and pooled in equimolar amounts. Here we consider only loci developed for C. maculata.

Genetic Identification Services (Chatsworth, California, USA) were provided with 100 µg of pooled genomic DNA, and enrichment and cloning were carried out as described previously (Jones et al., 2002). Concurrent partial digestion with RsaI, HaeIII, BstBI, PvuII, StuI, ScaI, and EcoRV was carried out and followed by adapter ligation. Biotinylated CA$_{(8)}$, GA$_{(8)}$, ATG$_{(8)}$, and TAGA$_{(8)}$ oligonucleotides were used for enrichment and capture of 300–750 bp fragments on magnetic beads (CPG, Lincoln Park, New Jersey, USA). Digestion with HindIII removed the adapters and was followed by ligation in pUC19 and transformation of DH5α cells. One colony each for motif (4) was sent to SeqWright (Houston, Texas, USA) for colony picking and insert sequencing.

In total, 768 colonies were sequenced. We designed primers for promising sequences using PRIMER3 (Rozen and Skaletsky, 2000) with an optimum primer length of 23 and $T_m$ of 62°C. The origins of prospective loci were determined via amplification from seven DNAs (three of which were individuals of C. maculata) representing the five orchid species (Lombardo et al., 2008). Amplification was attempted using an initial denaturation at 96°C for 2 min followed by 35 cycles of 92°C for 20 s, 58°C for 30 s, and 72°C for 30 s with a final elongation at 72°C for 10 min, with a total reaction volume of 10 µL containing 0.25 µL JumpStart REDTaq polymerase (Sigma-Aldrich, St. Louis, Missouri, USA), 200 µM dNTPs, 2.15 mM MgCl$_2$, 10 mM Tris-HCl, 50 mM KCl, and 0.001% gelatin (w/v).

 Primer pairs that successfully amplified C. maculata DNA were further screened, including bidirectional sequencing to verify the presence of a microsatellite.

Twenty-one individuals of C. maculata from the Colorado Rocky Mountains (San Juan NF: 37°31′N, 107°53′W, elevation 2017 m), 20 individuals of C. maculata from coastal California (37°52.16′N, 122°31.66′W, elevation 45 m), and six individuals of C. maculata from interior California (Mendocino NF: 31.66°N, 122°31.68°W, elevation 45 m), and six individuals of C. maculata from interior California (Mendocino NF: 3°31.68°W, elevation 45 m) were tested on three populations (Coastal California, Interior Northern California, and the Rocky Mountains in Colorado). The loci were also tested on a population of C. mertensiana, which is the only other widespread species within the C. maculata complex.
The significant deviations from Hardy–Weinberg equilibrium in all populations of *C. maculata* can be expected because of the highly selfing nature of the orchid (Freudenstein, 1997). Selfing and distinct lineages may also underlie the significant linkage disequilibrium in *C. maculata*, which was also found in the single nucleotide polymorphism study of Taylor et al. (2004). These new polymorphic microsatellite loci clearly delineate genetic variation and will aid in investigating the extent of gene flow taking place between populations and races of *C. maculata*. The cross-amplification of *C. mertensiana* will serve to increase our understanding of population dynamics in this species as well.

**LITERATURE CITED**


**Table 2.** Results of initial primer screening on samples from three populations of *Corallorhiza maculata* and one population of *C. mertensiana*. Shown for each locus are the number of alleles (A), observed and expected heterozygosity (H_0 and H_e), and the P values for Hardy–Weinberg equilibrium (HWE) for each population.


