Isolation of Microsatellite Markers in a Chaparral Species Endemic to Southern California, Ceanothus megacarpus (Rhamnaceae)

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**Methods and Results:** Four SSR-enriched libraries were used to develop and optimize 10 primer sets of microsatellite loci containing either di-, tri-, or tetranucleotide repeats. Levels of variation at these loci were assessed for two populations of C. megacarpus. Observed heterozygosity ranged from 0.250 to 0.885, and number of alleles ranged between four and 21 per locus. Eight to nine loci also successfully amplified in three other species of Ceanothus.

**Conclusions:** These markers should prove useful for evaluating the influence of recent and historical processes on genetic variation in C. megacarpus and related species.

Key words: Ceanothus; chaparral; microsatellites; Rhamnaceae.

Ceanothus megacarpus Nutt. (Rhamnaceae) is a diploid perennial shrub endemic to both coastal regions of the southern California Floristic Province and multiple Channel Islands off the coast of California (Fross and Wilken, 2006). This species has experienced considerable range fragmentation as a result of several factors including urbanization, increased fire frequency, and episodic droughts (Schlesinger and Gill, 1978; Witter et al., 2007). As a nonspouting species of chaparral shrub, C. megacarpus relies on the seedbank for recovery from fire, which initiates seed germination. Nevertheless, this species tends to disappear in areas of high fire frequency (Witter et al., 2007), such as seen in the Santa Monica Mountains, thus creating a mosaic of fragmented stands of C. megacarpus that differ in both age and density. Such fragmentation can have potential genetic consequences, including loss of genetic variation within fragmented stands and a decrease in gene flow between isolated fragments (Young et al., 1996). Interspecific hybridization is another potential factor that can influence patterns of genetic variation within species of Ceanothus L. (California lilac). Although introgressive hybridization between species within each subgenus (Cerastes and Ceanothus) has been documented, it is presumably rare between members of different subgenera (McMinn, 1942; Nobs, 1963).

Most genetic studies of Ceanothus have focused on phylogenetic relationships among species (Hardig et al., 2002; Burge et al., 2011), whereas little is known about genetic variation within and among species. Implementation of detailed genetic studies related to the effects of fragmentation and interspecific hybridization requires genetic markers variable enough to examine patterns of variation at the level of populations. Therefore, we developed a panel of microsatellite markers for C. megacarpus that is useful for detailed population studies and comparisons between species. This panel should prove useful for studies of some of the more dominant members of the chaparral shrub communities in California.

METHODS AND RESULTS

Tissue was collected from a single individual of C. megacarpus (34°02.395′N, 118°42.072′W) and sent to Genetic Identification Services (GIS; Chatsworth, California, USA). Genomic DNA was extracted, and four CA-, AAC-, ATG-, and TAGA-enriched libraries were developed using E. coli cells (strain DH5α) with the recombinant plasmid pUC19. Positive clones (n = 144) with inserts between 350 and 700 bp were sequenced, and 86 contained microsatellite loci. The criteria used to select clones for primer design included: (1) Microsatellite motifs were required to have enough flanking sequence for primer design. Clones containing microsatellite motifs near the ends of the sequence were excluded. (2) Flanking sequences had to meet standard design criteria. These included primer length (min = 18 bp, max = 22 bp), melting temperature...
null alleles, and corrected values of heterozygosity were obtained (Table 2). Micro-CHECKER, a potential explanation for the presence of null alleles and allele dropout was evaluated with MICRO-CHECKER version 2.2.3 (Van Oosterhout et al., 2004). Tests for HWE were performed separately for both populations of C. megacarpus (Table 2). CmegA125 and Cmeg121 revealed an excess of heterozygote frequencies (n = 55 °C, max = 60 °C), % GC (min = 35%, max = 65%), and PCR product length (min = 100 bp, max = 300 bp). Both DesignerPCR version 1.03 (Research Genetics, Huntsville, Alabama, USA) and Primer3Plus (Untergasser et al., 2007) were used to design primers for 20 unique loci, and these loci were selected based on the following criteria: (1) demonstration of polymorphism; (2) production of fragment patterns allowing for accurate allele calling; and (3) consistent PCR amplification. Genetic variation was assessed for two populations differing in the alleles present at individual loci. Although significant deviation from HWE was not observed for CmegA125, MICRO-CHECKER did suggest the presence of null alleles in population 1. Deviations from HWE for CmegA125, CmegB126, and Cmeg121 were also observed when both populations were combined, but this is likely the result of these two populations differing in the alleles present at individual loci. Although no detailed analysis was conducted, we did test to see if these microsatellite markers could prove useful for genetics studies of other species in the genus Ceanothus (Table 2). These species were selected based on their contrasting distribution patterns (Appendix 1): (1) one similar to C. megacarpus (C. crassifolius Hook.); (2) a more cosmopolitan species, C. cuneatus (Hook.) Nutt.; and (3) C. arboreus Greene, a species restricted to California islands. Ceanothus arboresus occurs in the subgenus Ceanothus, whereas all other species are in the subgenus Cerastes. Eight of the 10 loci consistently amplified across these species, and CmegA110 also amplified in C. arboresus. Although no data are shown, a preliminary study suggests that most of these loci, with the exception of CmegB8 and CmegB107, can be amplified across these species, and CmegA110 also amplified in C. arboresus. Although no detailed analysis was conducted, we did test to see if these microsatellite markers could prove useful for genetics studies of other species in the genus Ceanothus (Table 2). These species were selected based on their contrasting distribution patterns (Appendix 1): (1) one similar to C. megacarpus (C. crassifolius Hook.); (2) a more cosmopolitan species, C. cuneatus (Hook.) Nutt.; and (3) C. arboreus Greene, a species restricted to California islands. Ceanothus arboresus occurs in the subgenus Ceanothus, whereas all other species are in the subgenus Cerastes. Eight of the 10 loci consistently amplified across these species, and CmegA110 also amplified in C. arboresus. Although no data are shown, a preliminary study suggests that most of these loci, with the exception of CmegB8 and CmegB107, can be amplified for C. ferri- tiae McMin, a species occurring in serpentine soils. Given the low level of phylogenetic divergence within each subgenus (Burge et al., 2011), these markers should prove useful for detailed genetic studies of many species in both subgenera. Although significant deviation from HWE was not observed for CmegA125, MICRO-CHECKER did suggest the presence of null alleles in population 1. Deviations from HWE for CmegA125, CmegB126, and Cmeg121 were also observed when both populations were combined, but this is likely the result of these two populations differing in the alleles present at individual loci. Although no detailed analysis was conducted, we did test to see if these microsatellite markers could prove useful for genetics studies of other species in the genus Ceanothus (Table 2). These species were selected based on their contrasting distribution patterns (Appendix 1): (1) one similar to C. megacarpus (C. crassifolius Hook.); (2) a more cosmopolitan species, C. cuneatus (Hook.) Nutt.; and (3) C. arboreus Greene, a species restricted to California islands. Ceanothus arboresus occurs in the subgenus Ceanothus, whereas all other species are in the subgenus Cerastes. Eight of the 10 loci consistently amplified across these species, and CmegA110 also amplified in C. arboresus. Although no data are shown, a preliminary study suggests that most of these loci, with the exception of CmegB8 and CmegB107, can be amplified for C. ferri- tiae McMin, a species occurring in serpentine soils. Given the low level of phylogenetic divergence within each subgenus (Burge et al., 2011), these markers should prove useful for detailed genetic studies of many species in both subgenera.

CONCLUSIONS

We developed 10 microsatellite markers for the native California chaparral shrub, C. megacarpus, and have demonstrated that these loci are polymorphic across two populations. These markers are currently being used to investigate population structure, patterns of gene flow, and fragmentation of C. megacarpus in the Santa Monica Mountains of southern California. In addition, we confirmed cross-amplification of these microsatellite loci in additional species from both subgenera. Therefore, these markers will be highly advantageous for performing comparative studies on genetic diversity in Ceanothus with the potential application to more than 50 species from Baja California, California, and Oregon.
### Table 2. Patterns of variation observed across the 10 loci (CmegA4 to CmegD3) for *Ceanothus megacarpus*, *C. cuneatus*, *C. crassifolius*, and *C. arboreus*.

| Locus   | A (n=27) | Amo (n=25) | Merged (n=52) | Size range (bp) |
|---------|----------|------------|---------------|-----------------|
| CmegA4  | 16 0.852 | 17 0.824   | 11 0.868      | 285–299         |
| CmegA9  | 5 0.680  | 3 0.680    | 5 0.680       | 259–267         |
| CmegA10 | 4 0.519  | 4 0.519    | 4 0.519       | 277–283         |
| CmegA11 | 5 0.481  | 5 0.481    | 6 0.481       | 247–255         |
| CmegA12 | 4 0.519  | 4 0.519    | 4 0.519       | 277–283         |
| CmegB10 | 4 0.519  | 4 0.519    | 4 0.519       | 277–283         |
| CmegB11 | 5 0.481  | 5 0.481    | 6 0.481       | 247–255         |
| CmegB12 | 4 0.519  | 4 0.519    | 4 0.519       | 277–283         |
| CmegC10 | 9 0.630  | 10 0.630   | 10 0.630      | 277–283         |
| CmegC11 | 4 0.519  | 4 0.519    | 4 0.519       | 277–283         |
| CmegC12 | 7 0.556  | 8 0.556    | 8 0.556       | 277–283         |
| CmegC13 | 13 0.928 | 13 0.928   | 13 0.928      | 277–283         |
| CmegD3  | 13 0.928 | 13 0.928   | 13 0.928      | 277–283         |

Note: *A* = number of alleles; *He* = mean observed heterozygosity; *Ho* = mean expected heterozygosity; *n* = sample size.

Values in parentheses represent observed and expected heterozygosities obtained after genotypes were adjusted for potential null alleles based on the Oosterhout correction algorithm (Van Oosterhout et al., 2004). Asterisks (*) denote loci that were not in Hardy-Weinberg equilibrium: *P* < 0.05, **P** < 0.01, ***P*** < 0.001.

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APPENDIX 1. Voucher data for examined specimens of *Ceanothus*. The individual chaparral shrubs were marked with a metal tag, and stem, bud, and leaf samples were removed and are being maintained in a −70°C freezer. Each voucher specimen was given a separate number, and a GPS coordinate was recorded. All vouchers are deposited at Pepperdine University.

*C. megacarpus*: Cm6–152–178, Santa Monica Mountains, Los Angeles Co., Malibu, CA, USA (*n* = 27; 34°04.888′N, 118°45.513′W). *C. megacarpus*: Cm7–101–125, Santa Monica Mountains, Los Angeles Co., Malibu, CA, USA (*n* = 25; 34°04.856′N, 118°45.959′W). *C. cuneatus*: Ccu10-75–78, Ccu10-81–86, Santa Monica Mountains, Los Angeles Co., Malibu, CA, USA (*n* = 10; 34°05.949′N, 118°44.564′W). *C. crassifolius*: Ccr7-126–135, Santa Monica Mountains, Los Angeles Co., Malibu, CA, USA (*n* = 10, 34°04.856′N, 118°45.959′W). *C. arboreus*: Ca21–30, Santa Catalina Island, Los Angeles Co., CA, USA (*n* = 10; 33°23.295′N, 118°24.246′W).