

**ISOLATION AND CHARACTERIZATION OF MICROSATELLITE LOCI FOR CORNUS SANGUINEA (CORNACEAE)**

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**Premise of the study:** To facilitate genetic and conservation research of *Cornus sanguinea*, microsatellite loci were isolated and 29 individuals from 11 German populations were genotyped.

**Methods and Results:** Sixteen microsatellite loci were characterized from an enriched small insert genomic library. The number of alleles detected ranged from five to 11 per locus, observed heterozygosity ranged from 0.00 to 1.00, expected heterozygosity ranged from 0.65 to 0.90, and polymorphic information content ranged from 0.59 to 0.88.

**Conclusions:** The markers described in the study will allow further investigation of population dynamics and the degree of clonal reproduction within populations of *C. sanguinea*.

**Key words:** bloodtwig dogwood; Cornaceae; *Cornus sanguinea*; population genetics.

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*Cornus sanguinea* L. (Cornaceae) is a 4–5 m tall shrub (Liesebach and Götz, 2008) and is distributed over almost the entire European continent and in Asia in the Caucasus Mountains, northern Iran, Turkey, Syria, and Lebanon (see distribution map in Liesebach and Götz, 2008). *Cornus sanguinea* grows from lowlands to 1500 m in the Alps (Schütt et al., 1994). It can form hedges and grows along forest edges, riversides, and in floodplain forests and is often planted as an ornamental in cities and along roads. Genetic analysis of the species, based on isozymes (Leinemann et al., 2002) and chloroplast markers (Liesebach and Götz, 2008), was conducted to assess genetic variation in natural populations and to address conservation issues. Isozyme analyses were conducted on a small scale and revealed that most of the specimens within one natural shrub community were the result of vegetative reproduction (Leinemann et al., 2002). Previous studies have analyzed chloroplast markers for a panel of range-wide samples detecting lower genetic variation in *C. sanguinea* compared to other European tree species (e.g., *Fraxinus excelsior* L. [Heuertz et al., 2004]; *Corylus avellana* L. [Palmé and Vendramin, 2002]), with the latter study focusing mainly on large-scale differentiation in Europe. Small-scale genetic differentiation should be determined by analyzing population genetic research is essential to increase the productivity of the selected clones. Understanding the genetic composition of the selected clones is necessary, especially for clonally reproducing shrub and tree species such as *C. sanguinea*. Polymorphic nuclear microsatellites can establish and determine the degree of clonal reproduction in natural populations.

**METHODS AND RESULTS**

Samples of *C. sanguinea* were collected from 11 distinct locations in Germany, and representative voucher specimens were deposited at the Botanische Staatsammlung München (Appendix 1), Munich, Germany. For isolation of microsatellites, we followed protocols previously described by Wang et al. (2007) and Wadl et al. (2011). Briefly, genomic DNA (2.5 μg) was digested with the restriction enzymes *Alul*, *HaeIII*, and *RsaI* (New England BioLabs, Beverly, Massachusetts, USA) and ligated to SNX linker adapters (Hamilton et al., 1999). To enrich for sequences containing microsatellites, the SNX-ligated fragments were hybridized to (GT)\(_12\) primers (Vector, Glen Burnie, Maryland, USA) and transformed into *Escherichia coli* TOP10 cells (Invitrogen, Carlsbad, California, USA). PCR screening to select positive clones was performed using the following reaction: 1× GeneAmp PCR Buffer (Applied Biosystems, Carlsbad, California, USA), 2.5 mM MgCl\(_2\), 0.25 mM dNTPs, 0.25 μM T3 primer, 0.25 μM T7 primer, 0.25 μM (GT)\(_{12}\) primer, 0.3 U AmpliTaq Gold DNA Polymerase (Applied Biosystems), and sterile water. The reaction mixtures were PCR amplified using the following conditions: one cycle at 95°C for 3 min; 35 cycles at 95°C for 1 min, 50°C for 1 min, 72°C for 1 min; and one cycle at 72°C for 1 min. Clones that exhibited a smear when separated on 2% agarose gels were considered as positive for a microsatellite, and positive colonies (n = 192) were sequenced using universal T3 and T7 primers (Wang et al., 2007). Of the 192 clones sequenced, 148 (77.1%) contained microsatellites when searched using the default settings of the program Imperfect SSR Finder (Stieneke and Eujayl, 2007). Sequences were randomly

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1 Manuscript received 15 February 2013; revision accepted 1 May 2013. This work was supported by the United States Department of Agriculture (grant no. 58-6404-7-213). Mention of products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the authors.

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doi:10.3732/apps.1300012

Applications in Plant Sciences 2013 1(9): 1300012; http://www.bioone.org/loi/apps © 2013 Wadl et al. Published by the Botanical Society of America. This article is a U.S. Government work and is in the public domain in the USA.
Table 1. Characteristics of 16 microsatellite loci isolated from Cornus sanguinea.a

| Locus  | Primer sequences (5′–3′) | Repeat motif | Allelic class size range (bp) | \( T_c (°C) \) | GenBank accession no. |
|--------|--------------------------|--------------|-------------------------------|----------------|---------------------|
| CS4    | F: AAAGGAATGGCTTTGGTGAAT | (AC)\(_1\)   | 200–221                       | 52             | KC175472            |
|        | R: ACCTAGCAATGATGGGACTGTG |             |                               |                |                     |
| CS5    | F: AGCAATATGCAGAACACCTCAA | (TA)\(_2\)(TG)\(_1\) | 160–179                       | 52             | KC175473            |
|        | R: GTGCCCATTTGAGGAACAGGGA |             |                               |                |                     |
| CS6    | F: GTGAGTGTTTGAGGCTCTGTTT | (AC)\(_3\)(AT)(AC)\(_2\) | 217–230                       | 52             | KC175474            |
|        | R: GTGAGTGTTTGAGGCTCTGTTT |             |                               |                |                     |
| CS9    | F: CAAAGCTCTCTATCTCTGCCTGT | (TG)\(_1\)   | 234–249                       | 52             | KC175475            |
|        | R: GAGGCTGTAAAAGCCGAAATATA |             |                               |                |                     |
| CS15   | F: AACCCATTGGAACCCAGATACC | (CA)\(_4\)   | 149–163                       | 52             | KC175476            |
|        | R: TGTACATAGCTAAAGGGAGGAA |             |                               |                |                     |
| CS16   | F: GATGTTGACTATGTAGGCTGTT | (TG)\(_1\)   | 156–180                       | 52             | KC175477            |
|        | R: GCTAAAAAGGTTTAACAGATGGG |             |                               |                |                     |
| CS17   | F: TTGGGGATACCTGATGCTACAT | (TG)\(_1\)   | 211–257                       | 52             | KC175478            |
|        | R: AACACAGCAGATGGGAAATTTA |             |                               |                |                     |
| CS19   | F: CACAGAATCTGCTAGTACAAA | (TG)\(_1\)   | 135–182                       | 52             | KC175479            |
|        | R: GCTGATAAGGATCTGCTCTTT |             |                               |                |                     |
| CS21   | F: GATGATGATGATGATGATGATG | (TG)\(_1\)   | 182–217                       | 52             | KC175480            |
|        | R: CATTTGGTCTAAGGGATGTA |             |                               |                |                     |
| CS22   | F: AGAGGGATAGGCAGATGGTTA | (TG)\(_1\)   | 156–170                       | 52             | KC175481            |
|        | R: TGTGAGATTTAAGAGAGACACAT |             |                               |                |                     |
| CS24   | F: TGATTTCTCATATCCCCCTCTCT | (GT)\(_1\)(GT)\(_1\) | 181–211                       | 52             | KC175482            |
|        | R: CTCGAAATAGGGCGCAGT |             |                               |                |                     |
| CS25   | F: TGATTTCTCATATCCCCCTCTCT | (TG)\(_1\)   | 196–235                       | 52             | KC175483            |
|        | R: CCAACAGTGGCAACTAAAATCACA |             |                               |                |                     |
| CS26   | F: GGTGAGGGAAGGTTAGCTGTT | (TG)\(_1\)   | 138–176                       | 52             | KC175484            |
|        | R: TTGTACGATGACATGACATCTTA |             |                               |                |                     |
| CS27   | F: GTCACCTTTCAATGGTCAACA | (AC)\(_1\)   | 160–187                       | 52             | KC175485            |
|        | R: CACACCAATTTTTGAAAAACCAA |             |                               |                |                     |
| CS29   | F: GTCCCATCATATTTGGGACTGCT | (TG)\(_1\)   | 159–180                       | 52             | KC175486            |
|        | R: CGTGACATTGATGCTGCAATC |             |                               |                |                     |
| CS30   | F: ATTTGGATATACCACACATCCA | (CA)\(_3\)   | 151–177                       | 52             | KC175487            |
|        | R: TGTATGGGTAAACAGTGTTTTA |             |                               |                |                     |

Note: \( T_c \) = annealing temperature.

*a All values are based on 29 individuals from 11 populations in Germany (see Appendix 1).
CONCLUSIONS

Understanding the scales over which dispersal, genetic drift, and selection operate requires knowledge of population structure. There is a lack of knowledge of the genetic structure of *Cornus sanguinea*, in particular of clonal reproduction, which may exacerbate the effects of low gene flow by seed between populations. We expect that the microsatellites described in this study will be highly useful for population genetic studies and for assessing the degree of clonal reproduction in *C. sanguinea* in both natural populations and seed orchards.

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Appendix 1. *Cornus sanguinea* voucher specimens used in this study. All specimens are deposited at the Botanische Staatssammlung München (M), Munich, Germany.

| Collection no. | Locality (GPS coordinates) |
|---------------|---------------------------|
| BERT 1 (3)    | Bertholdsheim, Germany (48°43.984’N, 11°01.550’E) |
| BUCH 1 (3)    | Buch, Germany (48°38.317’N, 11°03.784’E) |
| DET 1 (3)     | Dettenheim, Germany (49°09.250’N, 8°25.417’E) |
| DOR 1 (3)     | Dorsbrunn, Germany (49°05.750’N, 10°54.167’E) |
| DOR 2 (3)     | Dorsbrunn, Germany (59°05.683’N, 10°55.000’E) |
| GEI 1 (3)     | Geichert, Germany (48°20.267’N, 11°34.000’E) |
| 09 1 (1)      | Holstein, Germany (53°55.984’N, 10°03.416’E) |
| 07 1 (1)      | Holstein, Germany (53°52.834’N, 9°46.117’E) |
| MAU 1 (3)     | Mauern, Germany (48°26.533’N, 11°05.100’E) |
| SCH 1 (3)     | Schnödhof, Germany (48°42.334’N, 11°04.350’E) |
| TRU 1 (3)     | Trugenhofen, Germany (48°46.084’N, 11°00.050’E) |