Validating Blackberry Seedling Pedigrees and Developing an Improved Multiplexed Microsatellite Fingerprinting Set

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Additional Index Words. Rubus, RosBREED, mixed-ploidy populations, phylogeny, Plant Variety Protection, plant identification, pedigree confirmation

Abstract. Confirming parentage and clonal identity is an important aspect of breeding and managing germplasm collections of clonally propagated, outcrossing crops, like blackberry (Rubus subgenus Rubus). DNA fingerprinting sets are used to identify off-cross progeny and confirm clonal identity. Previously, a six-simple sequence repeat (6-SSR) fingerprinting set was developed for blackberry using a small number of samples. The usefulness of the 6-SSR fingerprinting set for pedigree confirmation had not been evaluated. Therefore, it was used in this study to validate parentage for 6 and 12 biparental populations from the University of Arkansas (UA) and US Department of Agriculture Agricultural Research Service (USDA-ARS), Horticultural Crops Research Unit (HCRU) breeding programs, respectively. Twenty-seven of the 489 individuals in these breeding populations were identified as off-cross. The 6-SSR fingerprinting set was sufficient for parentage confirmation; however, a total of 61 plants distributed across 28 sets of genotypes could not be distinguished from each other. An 8-SSR fingerprinting set with improved resolution was subsequently developed and used to evaluate 177 Rubus accessions from the USDA-ARS National Clonal Germplasm Repository, UA, and USDA-ARS HCRU programs. The 8-SSR fingerprinting set distinguished all samples expected to have unique genotypes and identified differing DNA fingerprints for two sets of accessions suspected to have identical fingerprints. Cluster analysis grouped the accessions from the eastern and western US breeding programs based on geography and descent. Future work will focus on establishing a database of DNA fingerprints for germplasm identification and for determining pedigree relationships between blackberry accessions.

Blackberry is one of the many important commercialized fruit crops within the Rosaceae. The aggregate fruit produced by these plants have been enjoyed worldwide by people throughout history (Clark and Finn, 2011; Hummer and Janick, 2007). In North America, the majority of cultivars are poly-ploid, but the Rubus genus has a range of ploidy levels ranging from diploid to dodecaploid (Clark and Finn, 2011; Meng and Finn, 2002; Thompson, 1995). These cultivars often have complicated pedigrees consisting of many species from the genus. Except a few regionally important species, nearly all cultivated blackberries contain two or more Rubus species from four (Alleghenienis, Arguti, Rubus, and Ursini) of the 12 identified sections of the subgenus Rubus in their pedigrees (Clark and Finn, 2011; Watson, 1958). Clark et al. (2007) classified North American blackberry cultivars into two groups. The first group consists of erect and semierect blackberries (2n = 4x = 28) and trailing dewberries (2n = 2x = 14) domesticated from diploid and tetraploid species from eastern North America. The second group is comprised of trailing blackberries generated from polyploid species from western North America, such as Rubus ursinus (ploidy ranging from 8x to 12x), and introgressions of tetraploid blackberry and diploid red raspberry (R. idaeus, Idaeobatus) through intersectional hybrids [e.g., ‘Logan’ (2n = 6x = 42), ‘Tayberry’ (2n = 6x = 42), ‘Boysenberry’ (2n = 7x = 49), and ‘Youngberry’ (2n = 7x = 49)]. Trailing cultivars are generally of higher ploidy than the tetraploid erect eastern North American cultivars and can be found with ploidy levels ranging from 6x to 10x and 12x, in addition to occasional aneuploids (Meng and Finn, 2002; Thompson, 1995). The identification of cultivars and validation of pedigree is an important aspect of blackberry breeding and maintaining

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Received for publication 13 June 2018. Accepted for publication 9 Aug. 2018. The authors would like to thank April Nyberg, Jill Bushakra, Harrison Schreiber, Mary Peterson, Melissa Clark, Jaime Willard, David Gilmore, Tralena Kay Buck, Dan Chapman, and staff at the University of Arkansas System Division of Agriculture Fruit Research Station for their technical support. This research was funded through the USDA National Institute of Food and Agriculture Specialty Crop Research Initiative project “RosBREED: Combining Disease Resistance and Horticultural Quality in New Rosaceous Cultivars” (2014-51181-22378) and by the USDA-ARS CRIS projects 2072-21000-049-00D and 2072-21220-003-00D.
a germplasm collection as cultivars are clonally propagated and hybridize readily with nearby plants (Clark and Finn, 2011). DNA fingerprinting sets have become a useful tool to identify cultivars, validate pedigrees, study population diversity, and protect breeders’ rights (Laurentin, 2009; Peace, 2017). Before the use of DNA-based techniques, cultivar identification, pedigree validation, and population diversity for many plants were assessed by comparing morphology (Gilbert et al., 1999; Laurentin, 2009). Morphological traits can be highly influenced by environmental conditions, and closely related individuals may appear phenotypically the same (Gilbert et al., 1999; Laurentin, 2009). Of existing DNA-based markers, simple sequence repeat (SSR) markers are the preferred markers for cultivar identification and parentage confirmation. SSRs are abundant in plant genomes, cost-effective to use, amenable to automated scoring with software, and can be amplified in multiplex PCR (Bassil et al., 2016; Peace, 2017; Weising et al., 2005). Moreover, SSRs are highly polymorphic even amongst full-siblings and can be used to identify parental and sibling relationships (Bassil et al., 2012). In Rubus, SSRs were first isolated from red raspberry (Graham et al., 2002, 2004, 2006), and shown to cross-amplify in blackberry (Bassil et al., 2010; Bradish et al., 2016; Stafne et al., 2005). Before 2008, a small number of SSRs were isolated from blackberry species including eight SSRs from \textit{R. alcefolius} (Amsellem et al., 2000), 15 from \textit{R. hochstetterorum} (Lopes et al., 2006), and eight from ‘Marion’ blackberry (Castillo et al., 2010). Lewers et al. (2008) generated expressed sequence tags (ESTs) from ‘Merton Thornless’ blackberry and predicted the presence of 940 polymorphic SSRs in the genome based on evaluating ‘APF-12’ and ‘Arapaho’ with 33 SSRs. Thousands of SSRs were also recently identified from transcriptome sequencing of ‘Loch Ness’ blackberry (Garcia-Seco et al., 2015). SSRs used for cultivar identification in blackberry predominately had dinucleotide motifs (Bassil et al., 2016; Castillo et al., 2010; Marulanda et al., 2007, 2012). These dinucleotide motif SSRs can exhibit various artifacts such as stutters, split peaks, and binning errors (Bassil et al., 2016; Pompanon et al., 2005; Weising et al., 2005). SSRs with nucleotide motifs greater than two often do not exhibit these artifacts (Weising et al., 2005) and are recommended for use in fingerprinting sets (Akin et al., 2016; Testolin and Cipriani, 2010).

Bassil et al. (2016) recently evaluated 15 trinucleotide-containing SSRs in 13 diverse blackberry cultivars and created a 6-SSR fingerprinting set that distinguished these cultivars. The Bassil et al. (2016) 6-SSR fingerprinting set had not been assessed for its ability to confirm parentage and the resolution of the set was unknown due to the small number of samples evaluated in the previous study. The objective of this study was to evaluate this 6-SSR fingerprinting set for its ability to confirm parentage and distinguish seedlings of breeding populations from the University of Arkansas and the USDA-ARS Horticultural Crops Research Unit breeding programs; to develop an improved fingerprinting set, if needed; and to evaluate the new set for germplasm identification in a subset of the USDA-ARS National Clonal Germplasm Repository (NCGR) blackberry collection.

Materials and Methods

Germlasm. Crosses were made in 2011, 2012, and 2013 to create 6 and 12 biparental populations at the UA and the HCRU programs, respectively (Table 1; Supplemental Table 1). These 520 individuals were evaluated with the 6-SSR fingerprinting set. The biparental populations and the parental germplasm from the UA were grown at the UA Fruit Research Station (Clarksville, AR). The seedlings and parental germplasm in the HCRU program were grown at Oregon State University’s North Willamette Research and Extension Center (Aurora, OR) and at the Lewis-Brown Farm (Corvallis, OR). Additionally, 71 \textit{Rubus} subgenus \textit{Rubus} accessions from the NCGR, HCRU, and UA and a subset of 106 individuals evaluated with the 6-SSR fingerprinting set were evaluated with the newly developed 8-SSR fingerprinting set (Supplemental Table 1).

DNA extraction. Young actively growing leaf tissue from the apex of primocanes was collected from the populations and

Table 1. Summary of the blackberry populations from the University of Arkansas (UA) and the USDA-ARS Horticultural Crops Research Unit (HCRU) breeding programs.

| Population | Origin | Female parent | Male parent | Total progeny (no.) | True F\textsubscript{1} progeny (no.) |
|------------|--------|---------------|-------------|---------------------|---------------------------------|
| A-1222     | UA     | A-2444T       | APF-238T    | 44                  | 32                              |
| A-1229     | UA     | A-2488        | Prime-Ark\textsuperscript{*} 45 | 44                  | 43                              |
| A-1236     | UA     | Prime-Ark\textsuperscript{*} Traveler | A-2453T     | 44                  | 44                              |
| A-1250     | UA     | A-2466T       | Osage       | 44                  | 44                              |
| A-1253     | UA     | A-2487T       | A-2418T     | 44                  | 44                              |
| A-1261     | UA     | Galaxy        | A-2454T     | 44                  | 41                              |
| ORUS 4534  | HCRU   | Half’s Beauty | ORUS 2635-1 | 23                  | 23                              |
| ORUS 4540  | HCRU   | Galaxy        | Prime-Ark\textsuperscript{*} 45 | 23                  | 22                              |
| ORUS 4547  | HCRU   | Ouachita      | ORUS 3295-2 | 5                   | 0                               |
| ORUS 4647  | HCRU   | ORUS 2532-1   | Obsidian    | 23                  | 23                              |
| ORUS 4650  | HCRU   | ORUS 3172-1   | ORUS 2841-1 | 23                  | 22                              |
| ORUS 4651  | HCRU   | ORUS 3185-3   | Onyx        | 23                  | 20                              |
| ORUS 4660  | HCRU   | Columbia Star | Black Diamond | 23                  | 23                              |
| ORUS 4665  | HCRU   | Columbia Sunrise | ORUS 2841-1 | 23                  | 23                              |
| ORUS 4674  | HCRU   | Ouachita      | ORUS 3683-1 | 18                  | 17                              |
| ORUS 4754  | HCRU   | Columbia Star | ORUS 2707-1 | 17                  | 17                              |
| ORUS 4772  | HCRU   | ORUS 3308-2   | Metolius    | 18                  | 18                              |
| ORUS 4773  | HCRU   | ORUS 3308-2   | ORUS 2707-1 | 6                   | 6                               |
their parents. In the event that individuals did not have actively growing primocanes, the youngest possible leaf material was harvested. For DNA extraction, 30–50 mg of tissue were arrayed into two 96-well plates either containing silica to desiccate the samples for UA samples or steel beads for OR-grown samples from the HCRU or NCGR collections. The UA samples were shipped overnight to the NCGR. After desiccating for 1 week, the samples were ground using a mixer mill (MM 301; Retsch International, Hann, Germany). For OR-grown samples, the samples were frozen in liquid nitrogen and kept at −80 °C until ready for extraction. Oregon-grown samples were ground with a mixer mill (MM 301) and kept frozen by immersing in liquid nitrogen before grinding and halfway through the grinding. For all samples, DNA was extracted using a modified DNA extraction protocol [Puregene; Qiagen, Hilden, Germany (Gilmore et al., 2011)]. DNA was quantified via spectrophotometry using a multilabel plate reader (Victor3V 1420; PerkinElmer, Waltham, MA) and diluted to 3 ng·µL⁻¹.

**PEDIGREE VALIDATION USING THE 6-SSR FINGERPRINTING SET.** The populations were evaluated with the 6-SSR fingerprinting developed by Bassil et al. (2016) (Table 2). Polymerase chain reaction (PCR) was conducted using a microsatellite PCR kit (Type-it, Qiagen) in 15-µL reactions consisting of 8.3 µL of 2× Type-it Multiplex PCR Master Mix (Qiagen), 1.5 µL of PCR grade water, 1.7 µL of primer mix, and 3.5 µL of DNA at a concentration of 3 ng·µL⁻¹. The primers were evaluated at a final concentration of 0.057 µM for the forward primer and 0.227 µM for the reverse and M13 primers. Thermocycling conditions were as previously described; however, the annealing temperature during the 10 touchdown cycles began at 62 °C decreasing by 1 °C per cycle to a temperature of 52 °C and the second annealing temperature was 52 °C. Upon successful amplification, PCR products were pooled and alleles were separated via capillary electrophoresis (CEQ 8000). The markers were evaluated for their ease of scoring, lack of artifacts, the ability to distinguish unique accessions, and allelic size range. WellRED-labeled (Beckman Coulter) forward primers were ordered from Integrated DNA Technologies (Coralville, IA) for the best performing SSRs and primer concentrations were optimized for the conditions described for the 6-SSR fingerprinting set.

**COMPARISON OF THE 6-SSR AND 8-SSR FINGERPRINTING SETS.** The 6-SSR and 8-SSR fingerprinting sets were compared with one another on a subset of 106 individuals that had been evaluated with both fingerprinting sets. For each marker, the number of observed alleles and observed genotypes were counted and Nei’s gene diversity (Nei, 1978) was calculated at the allelic and genotypic level using the “locus_table” command in “Poppr” (Kamvar et al., 2014, 2015). The mean alleles and genotypes per locus and mean Nei’s gene diversity were also calculated for each fingerprinting set.

**ASSESSMENT OF POPULATION STRUCTURE FOR THE 8-SSR FINGERPRINTING SETS.** Population structure was evaluated for the 8-SSR fingerprinting set by constructing a dendrogram using the R packages “ape” [version 5.0 (Paradis et al., 2004)] and “Poppr” [version 2.6.1 (Kamvar et al., 2014, 2015)]. Null alleles were coded as “NA” and unknown alleles (e.g., masking of null alleles or unknown dosage configurations) were coded as “000” using the “recode_polyploids” command (Supplemental Table 2) (Bruvo et al., 2004; Kamvar et al., 2014, 2015). The “bruvo.boot” command (Kamvar et al., 2014, 2015) was used to produce a neighbor joining tree with the “njs” algorithm from “ape” and Bruvo’s distance (Bruvo et al., 2004) assuming an infinite allele, allele-sharing model (Supplemental Table 2) (Metzger et al., 2016). Bootstrap support of 2000 permutations was used (Supplemental Table 2). Bruvo’s distance was chosen for its ability to calculate distances for mixed-ploidy populations (Bruvo et al., 2004). K-means clustering was also used to visualize the data. When identifying the number of clusters for K-means clusters, the maximum number of principal components were retained and the number of clusters were selected based on the lowest Bayesian information criterion value.

**Results**

**PEDIGREE VALIDATION USING THE 6-SSR FINGERPRINTING SET.** The 6-SSR fingerprinting set was able to confirm parentage for the majority of the offspring (Table 1; Supplemental Table 1). Of the 489 progeny, 462 individuals (94.5%) were found to be true F₁s. Three of the six UA populations and five of the 12 HCRU populations had off-cross progeny (Table 1). All five individuals from the HCRU population ORUS 4547 were off-cross with the male parent being unknown. All progeny could be distinguished from one another for the UA population A-1222 and the HCRU populations ORUS 4547, ORUS 4647,
| Name                        | 8-SSR concn (µM) | Forward sequence | Reverse sequence                     | Source                              |
|-----------------------------|------------------|------------------|--------------------------------------|-------------------------------------|
| ERubLSQ_07-4_D05           | 0.113            | CTTCTTTCCAACCAGATTTC | GTTTACGAATTGATTTTCATCAACC           | Woodhead et al., 2008               |
| RH_MEa0003dF05              | 0.043            | TCCCCGGTCTACATATTCCA | GTCTGGCAATGGGAGCAGTT               | Lewers et al., 2008                 |
| RH_MEa0006bG05              | 0.028            | GAAAGCAGCAAGCAAGACCTTT | GTTTGTTCAAGGCAATGCTA               | Lewers et al., 2008                 |
| RH_MEa0007aG06              | 0.170            | AGATTGGAATTCAGGAATT | GTGCGTCAATCTCCATTG                 | Lewers et al., 2008                 |
| RH_MEa0011dG03a             | 0.085            | CCCTCACTCTCTCCATGCTC | CCAATTTCTGCAGGTTTGT                | Lewers et al., 2008                 |
| RH_MEa0013aC12              | 0.021            | TCCATCTCATCTCCAGGAACGT | GTGATGACGGTGGATGGACAG             | Lewers et al., 2008                 |
| RH_MEa0013dA06              | 0.340            | TGGGAGTGGAGAAGACAAGGG | ATTAATGACCCAGCCTCCTCC             | Lewers et al., 2008                 |
| RH_MEa0016aD11              | 0.085            | TACACCTATGCTCTCCTGAG | TTCAATTTGCTCTTCTGTCG              | Lewers et al., 2008                 |
| RH_MEa0016bC11              | 0.028            | CAGGAAGAAGCTGGTGT    | AACCCTGACACCTGCTCAATG             | Lewers et al., 2008                 |
| Ri11795                     | 0.013            | ATACATTCATTTGCTTCTCT | GCCGAGCAATGGAAGATGAAAT             | Dossett et al., 2015                |
| Ri06527                     | 0.043            | TATGATGCATCCATATCTGCT | GCTCTGAGTAATGGGGTATTCTCC         | Dossett et al., 2015                |
| Ro4261                      | 0.340            | AATAGCATGAGAATCCACTCA | TGGTATCTGCAAGGTGTTATCA            | Dossett et al., 2015                |
| Ro4532                      | 0.028            | ATACATGCGGAGAATGCTCTT | GCTCTGAGTAACTGGCTCCACG             | Dossett et al., 2015                |
| Ro942                       | 0.028            | AATGCACTGCTGCAAATATTC | CAATTTGACACACCTACG             | Dossett et al., 2015                |
| Rubleaf97                   | 0.028            | AACAAGCTGCCAGCAGGA | GTTGTGATGCTAATGGGAGCTG            | Graham et al., 2004                 |
| RhM011                      | 0.028            | AAGGAAAGGAGGAGCTGCTTACAC | GCTTTGATGCTTGAATGGGAGCTG     | Castillo et al., 2010               |
| RhM003                      | 0.028            | CCAATTCATCTCATGCTTCC | GCTTTGATGCTTGAATGGGAGCTG     | Castillo et al., 2010               |
| RiM019                      | 0.028            | ATCAAGAGCTTTAATGTCG  | GTTGTGATGCTAATGGGAGCTG           | Castillo et al., 2010               |
ORUS 4660, ORUS 4674, ORUS 4754, ORUS 4772, and ORUS 4773. In the remaining populations, a total of 61 individuals (49 from UA and 12 from HCRU) were distributed across 28 groups of individuals with identical fingerprints. The individuals that could not be differentiated consisted mostly of progeny; however, the parents A-2488 and ‘Prime-Ark Trav- erler’ were found to have identical fingerprints to the F1s A-1229-15 and A-1236-51, respectively.

**Creation of an improved 8-SSR fingerprinting set.** The 6-SSR fingerprinting set was improved upon to create an 8-SSR fingerprinting set by evaluating the 61 undifferentiated samples and population parents with 15 new SSRs (Table 2). The SSRs RH_MEa0003df05, RH_MEa0013bc12, Ri5037, Ro4261, Ro4532, Ro6594, and RiM019 were not able to differentiate any of the individuals with identical fingerprints. Ro942 and RH_MEa0015ce06 amplified well, were easy to score, and were highly polymorphic. Of the 28 groups of individuals with identical fingerprints, 10 groups could be distinguished using Ro942 and 11 groups could be distinguished with RH_MEa0015ce06. The allele ranges for these primer pairs ranged from 100 to 180 bp for Ro942 and 225 to 275 bp for RH_MEa0015ce06. The ranges of these primers could be accommodated through the use of WellRed dyes, which were not used for other primers in these ranges in the 6-SSR set. RH_MEa0008cf01 was able to further differentiate one group which could not be differentiated by either Ro942 or RH_MEa0015ce06. The allelic range of RH_MEa0008cf01 was also between 100 and 180 bp. The upper range of Ro942 and RH_MEa0008cf01 overlapped slightly with the lower range of RH_MEa0016bc11; however, both Ro942 and RH_MEa0008cf01 were more polymorphic. Therefore, RH_MEa0016bc11 was removed to avoid confusion when identifying alleles. After RH_MEa0016bc11 was removed, a non-specific 200-bp amplicon was not detected in subsequent multiplex reactions. This non-specific amplicon appeared to be the result of an off-target amplification with RH_MEa0007ag06 or RH_MEa0011dg03a. In conjunction, RH_MEa0008cf01, RH_MEa0015ce06, and Ro942 were able to resolve all but 10 sets of two individuals. One more set of individuals, ORUS 4540U and ORUS 4540T, could be further differentiated using the SSRs RiM019 or Rubleaf97. Unfortunately, the allelic ranges of these markers prevented them from being integrated into a single multiplex reaction as all dye combinations were already in use. WellRed-labeled forward primers were ordered for RH_MEa0008cf01, RH_MEa0015ce06, and Ro942. The primer concentrations for the 8-SSR assay were optimized for 15 μL reactions with identical components and thermocycling conditions to 6-SSR fingerprinting set (Table 2; Fig. 1) (Bassil et al., 2016).

**Comparison of the 6-SSR and 8-SSR fingerprinting sets.** A subset of 106 samples that were genotyped with both fingerprinting sets, consisting of 63 individuals from the UA breeding program and 43 individuals from the USDA-ARS HCRU breeding program, was used to compare the 6-SSR and 8-SSR fingerprinting sets (Supplemental Table 1). The average number of alleles, average number of genotypes, and Nei’s 1978 gene diversity was higher for the 8-SSR fingerprinting set than the 6-SSR fingerprinting set (Table 3) (Nei, 1978). RH_MEa0016bc11 had the second lowest gene diversity when calculated based on the alleles and the third lowest when calculated based on genotypes (Table 3). As such, RH_MEa0016bc11 was removed to make the 8-SSR fingerprinting set. ERubLRSQ_07-4_D05 performed the worst of all of the SSRs tested, with only three observed alleles, and was not polymorphic in the UA breeding program germplasm (Table 3). ERubLRSQ_07-4_D05 is a good candidate for replacement in the set. More work is needed to identify a suitable replacement within the 225 to 275-bp allelic range of ERubLRSQ_07-4_D05. The 8-SSR fingerprinting set performed better than the 6-SSR fingerprinting set when evaluating the two fingerprinting sets on subsets of germplasm from the UA and HCRU breeding programs (Table 3). A greater number of alleles and higher allelic and genotypic diversity parameters were observed in the HCRU subset than in the UA subset.

**Evaluation of the 8-SSR fingerprinting set in diverse germplasm.** A total of 177 accessions were evaluated with the 8-SSR fingerprinting set, consisting of 79 accessions from UA and other eastern North American breeding programs, 66 accessions from the HCRU breeding program, four accessions from Europe, one accession from Brazil, and 27 *Rubus* subgenus *Rubus* species, most of which are preserved at the NCGR (Supplemental Table 1). Within the species germplasm, 14 accessions originated from eastern North America, eight originated from western North America, and the remaining five originated from Spain, Russia, Pakistan, Turkey, and Montenegro. Six accessions (‘Columbia Star’, ‘Galaxy’, ‘Ki-owa’, ‘Loch Ness’, ‘Marion’, and ‘Prime Ark® 45’) from different sources (UA, HCRU, or NCGR) were found to have identical fingerprints (Fig. 2). ‘Thornless Evergreen’ and ‘Everthornless’ were found to have the same fingerprint. This
is not unexpected as ‘Everthornless’ was derived by isolating the thornless epidermal layer from the periclinal chimera ‘Thornless Evergreen’ using tissue culture (McPheeters and Skirvin, 2000). Interestingly, two accessions of ‘Burbank Thornless’ (CRUB 250.001 and CRUB 1815.001) from different sources had different fingerprints but grouped together in the dendrogram. Moreover ‘Young Thornless’ (CRUB 2003.001) was substantially different from ‘Young’ (CRUB 131.001). As ‘Young Thornless’ is reported to be a thornless sport of ‘Young’ they would be expected to have identical fingerprints (Coyner et al., 2005). All remaining accessions, except for the 10 groups of siblings that could not be differentiated, were found to have different fingerprints. When counting the observed number of alleles, the observed genotypes, and calculating Nei’s 1978 genetic diversity, all of the SSRs performed well with the exception of ERubLRSQ_07-4_D05 (Table 4; Nei, 1978). ERubLRSQ_07-4_D05 continued to exhibit lower allelic and genotypic diversity than the other SSRs in the 8-SSR fingerprinting set when evaluating a more diverse set of germplasm.

Hierarchical clustering using a Bruvo’s distance neighbor-joining tree identified distinct groups that separated primarily according to geography and pedigree (Fig. 2). In general, these six groups include a clade of western North American germplasm; a clade of eastern North American species germplasm; a clade of cultivars with ‘Merton Thornless’ in their pedigree; a group of clades containing *Rubus* species not native to North America; and two groups of multiple clades containing germplasm that originates from or has a parent from the UA breeding program (Fig. 2). Only one accession, ‘Columbia Giant’ (CRUB 2694.001) originating from western North America and a full-sib relative of ‘Columbia Star’, grouped unexpectedly with eastern North American germplasm (Fig. 2). This was only observed using hierarchical clustering and not with K-means clustering. Geographical separation was also observed when conducting K-means clustering that identified eight

| FP set       | Locus                  | Observed alleles (no.) | Nei’s 1978 gene diversity (allelic) | Observed genotypes (no.) | Nei’s 1978 gene diversity (genotypic) |
|--------------|------------------------|------------------------|-------------------------------------|--------------------------|---------------------------------------|
| 6-SSR        | RH_MEa0016bC11         | 10                     | 0.72                                | 18                       | 0.82                                  |
| Both         | RH_MEa0007aG06         | 12                     | 0.88                                | 50                       | 0.96                                  |
| Both         | RH_MEa0013dA06         | 11                     | 0.84                                | 38                       | 0.94                                  |
| Both         | ERubLRSQ_07-4_D05      | 3                      | 0.44                                | 5                        | 0.44                                  |
| Both         | RH_MEa0006bG05         | 10                     | 0.81                                | 27                       | 0.80                                  |
| Both         | RH_MEa0011dG03a        | 10                     | 0.84                                | 25                       | 0.93                                  |
| 8-SSR        | Ro942                  | 16                     | 0.86                                | 41                       | 0.94                                  |
| 8-SSR        | RH_MEa0008cF01         | 9                      | 0.83                                | 34                       | 0.96                                  |
| 8-SSR        | RH_MEa0015cE06         | 12                     | 0.84                                | 40                       | 0.95                                  |

|               | All samples            |                        |                                     |                          |                                       |
|---------------|------------------------|------------------------|-------------------------------------|--------------------------|---------------------------------------|
|               | Observed alleles (no.) | Observed genotypes (no.) | Nei’s 1978 gene diversity (allelic) | Nei’s 1978 gene diversity (genotypic) |

| 6-SSR        | RH_MEa0016bC11         | 4                      | 0.65                                | 7                        | 0.67                                  |
| Both         | RH_MEa0007aG06         | 8                      | 0.81                                | 19                       | 0.90                                  |
| Both         | RH_MEa0013dA06         | 6                      | 0.74                                | 12                       | 0.86                                  |
| Both         | ERubLRSQ_07-4_D05      | 1                      | -                                   | 1                        | -                                     |
| Both         | RH_MEa0006bG05         | 4                      | 0.50                                | 7                        | 0.56                                  |
| Both         | RH_MEa0011dG03a        | 5                      | 0.74                                | 9                        | 0.86                                  |
| 8-SSR        | Ro942                  | 9                      | 0.73                                | 14                       | 0.86                                  |
| 8-SSR        | RH_MEa0008cF01         | 6                      | 0.81                                | 20                       | 0.94                                  |
| 8-SSR        | RH_MEa0015cE06         | 7                      | 0.77                                | 20                       | 0.91                                  |

|               | Mean 6-SSR             | Mean 8-SSR             |                                     |                          |                                       |
|---------------|------------------------|------------------------|-------------------------------------|--------------------------|---------------------------------------|
|               | 4.67                   | 5.75                   | 0.57                                | 9.17                     | 0.64                                  |

|                | Observed alleles (no.) | Observed genotypes (no.) | Nei’s 1978 gene diversity (allelic) | Nei’s 1978 gene diversity (genotypic) |
|---------------|------------------------|--------------------------|-------------------------------------|---------------------------------------|
| 6-SSR         | RH_MEa0016bC11         | 10                      | 0.77                                | 16                       | 0.88                                  |
| Both          | RH_MEa0007aG06         | 12                      | 0.90                                | 33                       | 0.99                                  |
| Both          | RH_MEa0013dA06         | 11                      | 0.88                                | 32                       | 0.99                                  |
| Both          | ERubLRSQ_07-4_D05      | 3                       | 0.62                                | 5                        | 0.78                                  |
| Both          | RH_MEa0006bG05         | 10                      | 0.88                                | 25                       | 0.97                                  |
| Both          | RH_MEa0011dG03a        | 10                      | 0.88                                | 20                       | 0.95                                  |
| 8-SSR         | Ro942                  | 15                      | 0.90                                | 29                       | 0.98                                  |
| 8-SSR         | RH_MEa0008cF01         | 9                       | 0.81                                | 20                       | 0.93                                  |
| 8-SSR         | RH_MEa0015cE06         | 11                      | 0.89                                | 24                       | 0.95                                  |

|                | Mean 6-SSR             | Mean 8-SSR             |                                     |                          |                                       |
|---------------|------------------------|------------------------|-------------------------------------|--------------------------|---------------------------------------|
|               | 9.33                   | 10.13                  | 0.82                                | 23.50                    | 0.94                                  |
clusters after retaining 120 principal components (Fig. 2). Cluster 3 contained germplasm from western North American breeding programs and was distinct from the eastern North American germplasm. More structure was observed within the eastern North American germplasm and multiple clusters existed (Fig. 2). Clusters 2 and 5 consisted mostly of eastern North American hybrid *Rubus* cultivars and progeny from biparental crosses where one parent originated from the UA breeding program. Clusters 1, 4, and 8 consisted primarily of species germplasm that originated from the eastern United States. Many of the cultivars found in these clusters had *R. trivialis* or *R. riograndis* in their pedigree. The two ‘Burbank Thornless’ accessions comprised cluster 6. Finally, cluster 7 was comprised primarily of species germplasm from various geographical locations and ‘Merton Thornless’ and its descendants (Fig. 2). Nine of the 17 accessions within cluster 7...
originated from outside of North America (Supplemental Table 1) and an additional five (‘Black Satin’ CRUB 151.001, ‘Chester Thornless’ CRUB 839.001, ‘Ebano’ CRUB 833.001, ORUS 3295-2, and ORUS 3683-1) accessions have European heritage through ‘Merton Thornless’ derivatives in their ancestry.

Table 4. Summary statistics for each marker in the 8-SSR fingerprinting set on the 177 blackberry accessions genotyped with 8-SSR fingerprinting set.

| Locus          | Observed alleles (no.) | Nei’s 1978 gene diversity (allelic) | Observed genotypes (no.) | Nei’s 1978 gene diversity (genotypic) |
|---------------|------------------------|-------------------------------------|--------------------------|---------------------------------------|
| Ro942         | 35                     | 0.91                                | 94                       | 0.97                                  |
| RH_MEa0007aG06| 19                     | 0.90                                | 93                       | 0.98                                  |
| RH_MEa0008cf01| 10                     | 0.84                                | 59                       | 0.97                                  |
| RH_MEa0013dA06| 16                     | 0.87                                | 75                       | 0.96                                  |
| RH_MEa0015cE06| 15                     | 0.87                                | 63                       | 0.97                                  |
| ERubLRSQ_07-4_D05 | 7                  | 0.55                                | 11                       | 0.58                                  |
| RH_MEa0006bG05| 21                     | 0.86                                | 59                       | 0.87                                  |
| RH_MEa0011dG03a| 12                  | 0.86                                | 65                       | 0.96                                  |
| Mean          | 16.88                  | 0.83                                | 64.88                    | 0.91                                  |

Discussion

The 6-SSR fingerprinting set (Bassil et al., 2016) was found to be sufficient for determining parentage for the UA and HCRU biparental populations (Table 1). The resolution of the 6-SSR fingerprinting set was lacking and individuals from 10 populations had identical DNA fingerprints. Most of the sets of identical individuals were found in the UA populations. The UA breeding program breeds exclusively tetraploid cultivars. Conversely, the ploidy of cultivars produced by HCRU program is known to range from tetraploid to decaploid with the majority thought to be hexaploid (Meng and Finn, 2002; Thompson, 1995). Higher ploidy would allow for more potential gamete combinations during meiosis from each parent resulting in progeny that can be distinguished. The newly developed 8-SSR fingerprinting set was able to differentiate many of the individuals with identical DNA fingerprints. The remaining 10 groups of two undifferentiated individuals consisted of individuals that were planted adjacent to one another in the field. It is likely that the same plant may have been sampled twice as plants could have intergrown in the field.

None of the true F1 samples re-genotyped with the 8-SSR fingerprinting set were found to be off-cross. The new markers included in the 8-SSR fingerprinting set were able to distinguish all but 10 of the 28 groups of individuals that could not be distinguished with the 6-SSR fingerprinting set. Of these 10 groups, only one (ORUS 4540U and ORUS 4540T) could be distinguished using the SSRs RiM019 and Rubleaf 97 that could not be incorporated into the fingerprinting set. Therefore, the 6-SSR set efficiently confirmed parentage and the 8-SSR set resulted in enhanced distinguishing ability over that provided by the 6-SSR set as illustrated by the higher diversity parameters (Table 3). This improved fingerprinting set also distinguished homonyms and synonyms and will be useful for efficient management of the blackberry collection and elimination of synonyms. As expected based on higher ploidy (Meng and Finn, 2002; Thompson, 1995), a greater number of alleles and genotypes and higher diversity estimates were observed in the HCRU subset than in the UA subset. ERubLRSQ_07-4_D05 had substantially lower genetic diversity than the other SSRs in the 8-SSR fingerprinting set and is a candidate for replacement during any future improvements. The lower genetic diversity of ERubLRSQ_07-4_D05 was not surprising given it was designed from a red raspberry EST (Woodhead et al., 2008). SSRs created from ESTs are often conserved across closely related species; however, they display lower polymorphism when compared with non-genic SSRs (Chabane et al., 2005; Varshney et al., 2005).

The varying ploidy levels of hybrid blackberries and the frequency with which these levels change can pose unique challenges when comparing samples using traditional phylogenetic approaches. The majority of distance calculations were devised for diploid organisms and do not account for varying ploidy or allelic dosage (Bruvo et al., 2004). To address these problems, Bruvo et al. (2004) developed a distance calculation that factored in microsatellite mutations assuming a slipped-strand mispairing model and genome additions and losses. The genome addition and loss components of Bruvo’s distance were not as applicable to hybrid blackberries as genomes are gained or lost from one generation to the next rather than over the course of thousands of years. Moreover, in its original form Bruvo’s distance could only accommodate SSR markers. A solution to this problem was proposed by Metzger et al. (2016) by setting the repeat length parameter of Bruvo’s distance to a value smaller than one nucleotide. By doing so, the model becomes an allele-sharing model similar to Prevosti’s absolute genetic distance while still accounting for varying ploidy levels (Metzger et al., 2016; Prevosti et al., 1975). The approach proposed by Metzger et al. (2016) can be further extended to experiments using single nucleotide polymorphisms by coding the nucleotides as 1, 2, 3, and 4, making it a valuable tool for studying the relationships of mixed-ploidy populations.

K-means clustering tended to group individuals by identity-by-descent and hierarchical clustering grouped individuals by identity-by-state (Fig. 2). This is exemplified by ‘Columbia Giant’ (CRUB 2694.001) grouping with eastern North American germplasm rather than with western North American germplasm when using hierarchical clustering but not K-means clustering (Fig. 2). The genotypes of the two accessions of ‘Burbank Thornless’ (CRUB 250.001 and CRUB 1815.001) were different for three of the eight markers evaluated. Interestingly, a common allele was observed for each of the differing SSRs for the two accessions of the diploid ‘Burbank Thornless’ (Thompson, 1995). As half of the alleles were shared, it is possible that one of the accessions is an offspring of the other or both accessions are half-sibling offspring of ‘Burbank Thornless’. The fingerprints of ‘Young Thornless’
ICRUB 2003.001) and ‘Young’ (CRUB 131.001) were substantially different at each locus tested. ‘Young Thornless’ was donated by a private grower in Washington State. It is likely that one of these accessions was misidentified. In the cases of ‘Burbank Thornless’ and ‘Young’, further work with additional collections of the respective accessions will be needed to fully resolve these differences and identify a true-to-type candidate. The dendrogram and K-means cluster show the HCRU program collections of the respective accessions will be needed to fully resolve these differences and identify a true-to-type candidate.

The 8-SSR fingerprinting set is an excellent tool for parentage and identity confirmation, as well as plant variety protection. Future work will focus on evaluating additional accessions within the NCGR blackberry germplasm collection to establish a large working library to aid in accession identification and diversity preservation. Additional work is needed to identify the correct accessions of ‘Burbank Thornless’, ‘Young’, and ‘Young Thornless’ and to assess the 8-SSR fingerprinting set’s performance in other Rubus subgenera.

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Supplemental Table 1. List of all blackberry accessions evaluated, the source of the accession, which fingerprinting set the accession was genotyped with, and the results of the parentage analysis for the six University of Arkansas (UA) populations and 12 USDA-ARS Horticultural Crops Research Unit (HCRU) populations. The accessions tested were collected from the UA, HCRU, or the USDA-ARS National Clonal Germplasm Repository (NCGR). The results of the parentage testing are also displayed for progeny tested with the 6-SSR fingerprinting set. Progeny listed as “true F1s” were identified to likely be the result of a cross between the suspected parents. Those listed as “off-cross” had at least one allele that could not be found in either of the suspected parents.

| Clone name   | Clone source | Accession origin | Fingerprinting set | Results of parentage analysis |
|--------------|--------------|-----------------|--------------------|-------------------------------|
| A-1222-1     | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-2     | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-3     | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-4     | UA           | Arkansas         | 6-SSR              | Off-cross                     |
| A-1222-6     | UA           | Arkansas         | 6-SSR              | Off-cross                     |
| A-1222-7     | UA           | Arkansas         | 6-SSR              | Off-cross                     |
| A-1222-9     | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-11    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-12    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-13    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-14    | UA           | Arkansas         | 6-SSR              | Off-cross                     |
| A-1222-15    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-16    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-18    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-20    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-21    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-23    | UA           | Arkansas         | 6-SSR              | Off-cross                     |
| A-1222-24    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-26    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-27    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-28    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-29    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-30    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-32    | UA           | Arkansas         | 6-SSR              | Off-cross                     |
| A-1222-33    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-34    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-35    | UA           | Arkansas         | 6-SSR              | Off-cross                     |
| A-1222-36    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-37    | UA           | Arkansas         | 6-SSR              | Off-cross                     |
| A-1222-38    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-39    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-40    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-41    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-42    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-44    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-45    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-46    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-47    | UA           | Arkansas         | 6-SSR              | Off-cross                     |
| A-1222-49    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-50    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-51    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1222-52    | UA           | Arkansas         | 6-SSR              | Off-cross                     |
| A-1229-2     | UA           | Arkansas         | Both               | True F1                       |
| A-1229-3     | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-4     | UA           | Arkansas         | Both               | True F1                       |
| A-1229-5     | UA           | Arkansas         | Both               | True F1                       |
| A-1229-7     | UA           | Arkansas         | Both               | True F1                       |
| A-1229-9     | UA           | Arkansas         | Both               | True F1                       |
| A-1229-10    | UA           | Arkansas         | 6-SSR              | Off-cross                     |
| A-1229-11    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-12    | UA           | Arkansas         | Both               | True F1                       |

Continued next page
## Supplemental Table 1. Continued.

| Clone name   | Clone source | Accession origin | Fingerprinting set | Results of parentage analysis |
|--------------|--------------|------------------|--------------------|-------------------------------|
| A-1229-13    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-14    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-15    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-16    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-17    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-18    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-19    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-20    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-22    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-23    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-24    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-25    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-26    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-27    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-28    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-29    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-30    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-31    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-32    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-33    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-34    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-35    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-36    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-37    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-38    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-39    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-40    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-41    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-42    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-43    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-44    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-45    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-46    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1229-47    | UA           | Arkansas         | Both               | True F1                       |
| A-1229-48    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-2     | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-5     | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-6     | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-9     | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-11    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-12    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-13    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-14    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-15    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-16    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-17    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-18    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-19    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-20    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-21    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-22    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-23    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-24    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-25    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-26    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-27    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-28    | UA           | Arkansas         | Both               | True F1                       |
| A-1236-29    | UA           | Arkansas         | Both               | True F1                       |
Supplemental Table 1. Continued.

| Clone name  | Clone source | Accession origin | Fingerprinting set | Results of parentage analysis |
|-------------|--------------|------------------|--------------------|-------------------------------|
| A-1236-30   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-31   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-32   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-33   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-34   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-35   | UA           | Arkansas         | Both               | True F1                       |
| A-1236-36   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-37   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-38   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-39   | UA           | Arkansas         | Both               | True F1                       |
| A-1236-40   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-41   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-42   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-43   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-44   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-45   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-46   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-47   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-48   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-49   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-50   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1236-51   | UA           | Arkansas         | Both               | True F1                       |
| A-1250-1    | UA           | Arkansas         | Both               | True F1                       |
| A-1250-2    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-3    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-4    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-5    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-6    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-7    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-8    | UA           | Arkansas         | Both               | True F1                       |
| A-1250-9    | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-10   | UA           | Arkansas         | Both               | True F1                       |
| A-1250-11   | UA           | Arkansas         | Both               | True F1                       |
| A-1250-12   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-13   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-14   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-15   | UA           | Arkansas         | Both               | True F1                       |
| A-1250-16   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-17   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-18   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-19   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-20   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-21   | UA           | Arkansas         | Both               | True F1                       |
| A-1250-22   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-23   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-24   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-25   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-26   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-27   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-28   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-29   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-30   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-31   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-32   | UA           | Arkansas         | Both               | True F1                       |
| A-1250-33   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-34   | UA           | Arkansas         | Both               | True F1                       |
| A-1250-35   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-36   | UA           | Arkansas         | Both               | True F1                       |
| A-1250-37   | UA           | Arkansas         | Both               | True F1                       |
| A-1250-38   | UA           | Arkansas         | Both               | True F1                       |
| A-1250-39   | UA           | Arkansas         | Both               | True F1                       |
| A-1250-40   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-41   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-42   | UA           | Arkansas         | 6-SSR              | True F1                       |
| A-1250-43   | UA           | Arkansas         | Both               | True F1                       |

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Supplemental Table 1. Continued.

| Clone name   | Clone source | Accession origin | Fingerprinting set | Results of parentage analysis |
|--------------|--------------|------------------|--------------------|-------------------------------|
| A-1250-44    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1250-45    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1250-46    | UA           | Arkansas          | Both               | True F1                       |
| A-1250-47    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1250-48    | UA           | Arkansas          | Both               | True F1                       |
| A-1250-49    | UA           | Arkansas          | Both               | True F1                       |
| A-1250-50    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1250-51    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1250-52    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-1     | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-2     | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-4     | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-6     | UA           | Arkansas          | Both               | True F1                       |
| A-1253-7     | UA           | Arkansas          | Both               | True F1                       |
| A-1253-8     | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-9     | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-10    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-11    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-12    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-13    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-14    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-15    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-17    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-19    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-21    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-23    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-24    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-25    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-26    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-27    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-28    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-29    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-30    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-31    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-32    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-33    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-34    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-35    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-36    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-37    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-38    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-39    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-40    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-41    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-42    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-43    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-44    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-45    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-46    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-47    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-48    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-49    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1253-50    | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1261-1     | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1261-2     | UA           | Arkansas          | 6-SSR              | True F1                       |
| A-1261-3     | UA           | Arkansas          | 6-SSR              | Off-cross                     |
| A-1261-4     | UA           | Arkansas          | 6-SSR              | True F1                       |

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| Clone name | Clone source | Accession origin | Fingerprinting set | Results of parentage analysis |
|------------|--------------|-----------------|-------------------|------------------------------|
| A-1261-5   | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-6   | UA           | Arkansas        | 6-SSR             | Off-cross                    |
| A-1261-7   | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-8   | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-9   | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-10  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-11  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-12  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-13  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-14  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-15  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-16  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-17  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-18  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-19  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-20  | UA           | Arkansas        | Both              | Off-cross                    |
| A-1261-21  | UA           | Arkansas        | Both              | True F1                      |
| A-1261-22  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-23  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-24  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-25  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-26  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-27  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-28  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-29  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-30  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-31  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-32  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-33  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-34  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-35  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-36  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-37  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-38  | UA           | Arkansas        | Both              | True F1                      |
| A-1261-39  | UA           | Arkansas        | Both              | True F1                      |
| A-1261-40  | UA           | Arkansas        | Both              | True F1                      |
| A-1261-41  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-42  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-43  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-44  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-45  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-46  | UA           | Arkansas        | Both              | True F1                      |
| A-1261-47  | UA           | Arkansas        | Both              | True F1                      |
| A-1261-48  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-1261-49  | UA           | Arkansas        | 6-SSR             | True F1                      |
| A-2418T    | UA           | Arkansas        | Both              |                             |
| A-2444T    | UA           | Arkansas        | Both              |                             |
| A-2453T    | UA           | Arkansas        | Both              |                             |
| A-2454T    | UA           | Arkansas        | Both              |                             |
| A-2466T    | UA           | Arkansas        | Both              |                             |
| A-2487T    | UA           | Arkansas        | Both              |                             |
| A-2488     | UA           | Arkansas        | Both              |                             |
| Apache CRUB 2000.001 | NCGR | Arkansas | 8-SSR |                       |
| APF-236T   | HCRU         | Arkansas        | Both              |                             |
| APF-238T   | UA           | Arkansas        | Both              |                             |
| Arapaho CRUB 1726.001 | NCGR | Arkansas | 8-SSR |                       |
| Bedford Thornless CRUB 857.001 | NCGR | United Kingdom | 8-SSR |                       |
| Black Butte CRUB 1861.001 | NCGR | Oregon | 8-SSR |                       |
| Black Diamond | HCRU | Oregon | Both |                             |
| Black Satin CRUB 151.001 | NCGR | Illinois | 8-SSR |                       |
| Burbank Thornless CRUB 1815.001 | NCGR | California | 8-SSR |                       |
| Burbank Thornless CRUB 250.001 | NCGR | California | 8-SSR |                       |

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Supplemental Table 1. Continued.

| Clone name                  | Clone source | Accession origin | Fingerprinting set | Results of parentage analysis |
|-----------------------------|--------------|------------------|--------------------|-------------------------------|
| Cascade CRUB66.001          | NCGR         | Oregon           | 8-SSR              |                               |
| Chehalem CRUB 761.001       | NCGR         | Oregon           | 8-SSR              |                               |
| Cherokee CRUB67.001         | NCGR         | Arkansas         | 8-SSR              |                               |
| Chester Thornless CRUB 839.001 | NCGR      | Illinois         | 8-SSR              |                               |
| Cheyenne CRUB68.001         | NCGR         | Arkansas         | 8-SSR              |                               |
| Chicasaw CRUB 1999.001      | NCGR         | Arkansas         | 8-SSR              |                               |
| Choctaw CRUB 1582.001       | NCGR         | Arkansas         | 8-SSR              |                               |
| Columbia Giant CRUB 2694.001 | NCGR     | Oregon           | 8-SSR              |                               |
| Columbia Star               | HCRU         | Oregon           | Both               |                               |
| Columbia Star CRUB 2643.001 | NCGR         | Oregon           | 8-SSR              |                               |
| Columbia Sunrise            | HCRU         | Oregon           | Both               |                               |
| Douglass CRUB 1416.002      | NCGR         | Oregon           | 8-SSR              |                               |
| Ebano CRUB 833.001          | NCGR         | Arkansas         | 8-SSR              |                               |
| Everthornless CRUB 2310.001 | NCGR         | Oregon           | 8-SSR              |                               |
| Galaxy                      | HCRU         | Oregon           | Both               |                               |
| Galaxy                      | UA           | Oregon           | Both               |                               |
| Hall’s Beauty               | HCRU         | Oregon           | Both               |                               |
| Hillquist CRUB 723.001      | NCGR         | Virginia         | 8-SSR              |                               |
| Jenner CRUB 137.002         | NCGR         | Oregon           | 8-SSR              |                               |
| Kiowa                       | HCRU         | Arkansas         | 8-SSR              |                               |
| Kiowa CRUB 1947.001         | NCGR         | Arkansas         | 8-SSR              |                               |
| Loch Ness CRUB 1863.001     | NCGR         | United Kingdom   | 8-SSR              |                               |
| Loch Ness CRUB 1863.002     | NCGR         | United Kingdom   | 8-SSR              |                               |
| Long Black CRUB 1942.001    | NCGR         | Oregon           | 8-SSR              |                               |
| Marion                      | HCRU         | Oregon           | Both               |                               |
| Marion CRUB 385.001         | NCGR         | Oregon           | 8-SSR              |                               |
| Merton Thornless CRUB 254.001 | NCGR     | United Kingdom   | 8-SSR              |                               |
| Metolius                    | HCRU         | Oregon           | Both               |                               |
| Navaho                      | HCRU         | Arkansas         | 8-SSR              |                               |
| Nettleton’s Creamy White CRUB 2658.001 | NCGR | Illinois         | 8-SSR              |                               |
| Newberry CRUB 2547.001      | NCGR         | Oregon           | 8-SSR              |                               |
| NZ 9337-1 CRUB 2343.001     | NCGR         | Oregon           | 8-SSR              |                               |
| NZ 9351-4 CRUB 2344.001     | NCGR         | Oregon           | 8-SSR              |                               |
| NZ 9373-1 CRUB 2345.001     | NCGR         | Oregon           | 8-SSR              |                               |
| NZ 9672-1 CRUB 2517.001     | NCGR         | Oregon           | 8-SSR              |                               |
| Obsidian                    | HCRU         | Oregon           | Both               |                               |
| Oklawaha CRUB 720.001       | NCGR         | Florida          | 8-SSR              |                               |
| Olallie CRUB 76.001         | NCGR         | Oregon           | 8-SSR              |                               |
| Onyx                        | HCRU         | Oregon           | Both               |                               |
| ORUS 1513-1                 | HCRU         | Oregon           | Both               |                               |
| ORUS 2532-1                 | HCRU         | Oregon           | 8-SSR              |                               |
| ORUS 2635-1                 | HCRU         | Oregon           | Both               |                               |
| ORUS 2707-1                 | HCRU         | Oregon           | Both               |                               |
| ORUS 2841-1                 | HCRU         | Oregon           | Both               |                               |
| ORUS 3172-1                 | HCRU         | Oregon           | Both               |                               |
| ORUS 3185-3                 | HCRU         | Oregon           | Both               |                               |
| ORUS 3295-2                 | HCRU         | Oregon           | Both               |                               |
| ORUS 3308-2                 | HCRU         | Oregon           | 8-SSR              |                               |
| ORUS 3681-1                 | HCRU         | Oregon           | Both               |                               |
| ORUS 3683-1                 | HCRU         | Oregon           | Both               |                               |
| ORUS 4534A                  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4534B                  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4534C                  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4534D                  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4534E                  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4534F                  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4534G                  | HCRU         | Oregon           | 6-SSR              | True F1                       |

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| Clone name | Clone source | Accession origin | Fingerprinting set | Results of parentage analysis |
|------------|--------------|------------------|--------------------|-------------------------------|
| ORUS 4534H| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4534I| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4534J| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4534K| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4534L| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4534M| HCRU         | Oregon           | Both               | True F₁                      |
| ORUS 4534N| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4534O| HCRU         | Oregon           | Both               | True F₁                      |
| ORUS 4534P| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4534Q| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4534R| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4534S| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4534T| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4540A| HCRU         | Oregon           | Both               | True F₁                      |
| ORUS 4540B| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4540C| HCRU         | Oregon           | Both               | True F₁                      |
| ORUS 4540D| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4540E| HCRU         | Oregon           | Both               | True F₁                      |
| ORUS 4540F| HCRU         | Oregon           | Both               | True F₁                      |
| ORUS 4540G| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4540H| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4540I| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4540J| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4540K| HCRU         | Oregon           | Both               | True F₁                      |
| ORUS 4540L| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4540M| HCRU         | Oregon           | Both               | True F₁                      |
| ORUS 4540N| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4540O| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4540P| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4540Q| HCRU         | Oregon           | Both               | True F₁                      |
| ORUS 4540R| HCRU         | Oregon           | Both               | True F₁                      |
| ORUS 4540S| HCRU         | Oregon           | Both               | True F₁                      |
| ORUS 4540T| HCRU         | Oregon           | Both               | True F₁                      |
| ORUS 4540U| HCRU         | Oregon           | Both               | True F₁                      |
| ORUS 4540V| HCRU         | Oregon           | Both               | True F₁                      |
| ORUS 4540W| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4547A| HCRU         | Oregon           | 6-SSR              | Off-cross                    |
| ORUS 4547B| HCRU         | Oregon           | 6-SSR              | Off-cross                    |
| ORUS 4547C| HCRU         | Oregon           | 6-SSR              | Off-cross                    |
| ORUS 4547D| HCRU         | Oregon           | 6-SSR              | Off-cross                    |
| ORUS 4547E| HCRU         | Oregon           | 6-SSR              | Off-cross                    |
| ORUS 4547F| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4547G| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4547H| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4547I| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4547J| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4547K| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4547L| HCRU         | Oregon           | 6-SSR              | True F₁                      |
| ORUS 4547M| HCRU         | Oregon           | 6-SSR              | True F₁                      |

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| Clone name | Clone source | Accession origin | Fingerprinting set | Results of parentage analysis |
|------------|--------------|------------------|--------------------|-------------------------------|
| ORUS 4647N| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4647O| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4647P| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4647Q| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4647R| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4647S| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4647T| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4647U| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4647V| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4647W| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650A| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650B| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650C| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650D| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650E| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650F| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650G| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650H| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650I| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650J| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650K| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650L| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650M| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650N| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650O| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650P| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650Q| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650R| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650S| HCRU         | Oregon           | Both               | True F1                       |
| ORUS 4650T| HCRU         | Oregon           | Both               | True F1                       |
| ORUS 4650U| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650V| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4650W| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651A| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651B| HCRU         | Oregon           | Both               | True F1                       |
| ORUS 4651C| HCRU         | Oregon           | Both               | True F1                       |
| ORUS 4651D| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651E| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651F| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651G| HCRU         | Oregon           | Both               | True F1                       |
| ORUS 4651H| HCRU         | Oregon           | Both               | True F1                       |
| ORUS 4651I| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651J| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651K| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651L| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651M| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651N| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651O| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651P| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651Q| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651R| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651S| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651T| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651U| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651V| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4651W| HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660A| HCRU         | Oregon           | 6-SSR              | True F1                       |

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| Clone name   | Clone source | Accession origin | Fingerprinting set | Results of parentage analysis |
|-------------|--------------|------------------|--------------------|-------------------------------|
| ORUS 4660B  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660C  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660D  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660E  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660F  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660G  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660H  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660I  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660J  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660K  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660L  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660M  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660N  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660O  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660P  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660Q  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660R  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660S  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660T  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660U  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660V  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4660W  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665A  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665B  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665C  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665D  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665E  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665F  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665G  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665H  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665I  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665J  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665K  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665L  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665M  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665N  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665O  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665P  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665Q  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665R  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665S  | HCRU         | Oregon           | Both               | True F1                       |
| ORUS 4665T  | HCRU         | Oregon           | Both               | True F1                       |
| ORUS 4665U  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665V  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4665W  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674A  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674B  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674C  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674D  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674E  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674F  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674G  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674H  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674I  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674J  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674K  | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674L  | HCRU         | Oregon           | 6-SSR              | True F1                       |

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Supplemental Table 1. Continued.

| Clone name       | Clone source | Accession origin | Fingerprinting set | Results of parentage analysis |
|------------------|--------------|------------------|--------------------|-------------------------------|
| ORUS 4674M       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674N       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674O       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674P       | HCRU         | Oregon           | 6-SSR              | Off-cross                     |
| ORUS 4674Q       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4674R       | HCRU         | Oregon           | Both               | True F1                       |
| ORUS 4754A       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754B       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754C       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754D       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754E       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754F       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754G       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754H       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754I       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754J       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754K       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754L       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754M       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754N       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754O       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754P       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754Q       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4754R       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772A       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772B       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772C       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772D       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772E       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772F       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772G       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772H       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772I       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772J       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772K       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772L       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772M       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772N       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772O       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772P       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772Q       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4772R       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4773A       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4773B       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4773C       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4773D       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4773E       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| ORUS 4773F       | HCRU         | Oregon           | 6-SSR              | True F1                       |
| Osage            | UA           | Arkansas         | Both               |                               |
| Ouachita         | HCRU         | Arkansas         | 6-SSR              |                               |
| Pacific CRUB 1220.001 | NCGR   | Oregon           | 8-SSR              |                               |
| Phenomenal CRUB 1817.001 | NCGR | California       | 8-SSR              |                               |
| Prime-Ark® 45    | HCRU         | Arkansas         | Both               |                               |
| Prime-Ark® 45    | UA           | Arkansas         | Both               |                               |
| Prime-Ark® Traveler | UA    | Arkansas         | Both               |                               |
| Prime-Jim        | UA           | Arkansas         | 8-SSR              |                               |
| _R. allegheniensis_ eastern upright CRUB 1781.001 | NCGR | Arkansas         | 8-SSR              |                               |
| _R. allegheniensis_ Pennsylvania CRUB 1152.001 | NCGR | Pennsylvania     | 8-SSR              |                               |

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Supplemental Table 1. Continued.

| Clone name          | Clone source | Accession origin  | Fingerprinting set | Results of parentage analysis |
|---------------------|--------------|-------------------|--------------------|-------------------------------|
| R. allegheniensis rual050 | CRUB 2098.001 | NCGR Southeast USA | 8-SSR              |                               |
| R. argutus NC 95-2-1 | CRUB 1818.001 | NCGR South Carolina | 8-SSR           |                               |
| R. caesius CRUB 149.003 | CRUB 27.001  | NCGR Russia       | 8-SSR              |                               |
| R. canadensis Georgia CRUB 817.001 | NCGR Georgia (USA) | 8-SSR |                               |
| R. canadensis NC 86-33-4 | CRUB 785.001 | NCGR North Carolina | 8-SSR          |                               |
| R. hispida 15 CRUB 1414.001 | NCGR Pennsylvania | 8-SSR |                               |
| R. hispida NC 86-42-4 | CRUB 794.001 | NCGR North Carolina | 8-SSR          |                               |
| R. pensilvanicus BWP CRUB 2606.001 | NCGR California | 8-SSR |                               |
| R. sanctus 880334 CRUB 1054.002 | NCGR Pakistan | 8-SSR |                               |
| R. trivialis M-26826 CRUB 260.001 | NCGR Georgia (USA) | 8-SSR |                               |
| R. ulmifolius Turkey CRUB 23.001 | NCGR Turkey | 8-SSR |                               |
| R. ulmifolius Yugo CRUB 34.001 | NCGR Montenegro | 8-SSR |                               |
| R. ursinus CRUB611.001 | NCGR Idaho    | 8-SSR              |                   |                               |
| Rio Grande CRUB 2053.001 | NCGR Louisiana | 8-SSR |                               |
| Santiam CRUB 79.001 | NCGR Oregon | 8-SSR              |                   |                               |
| Siskiyou CRUB 1387.001 | NCGR Oregon | 8-SSR              |                   |                               |
| Thornless Evergreen CRUB 991.001 | NCGR Oregon | 8-SSR |                               |
| Triple Crown CRUB 1946.001 | NCGR Maryland | 8-SSR |                               |
| Tupy CRUB 2192.001 | NCGR Brazil   | 8-SSR              |                   |                               |
| Waldo CRUB 983.001 | NCGR Oregon | 8-SSR              |                   |                               |
| Whitford Thornleaf CRUB 722.002 | NCGR Illinois | 8-SSR |                               |
| Wild Treasure CRUB 2237.001 | NCGR Oregon | 8-SSR |                               |
| Young CRUB 131.001 | NCGR Louisiana | 8-SSR |                               |
| Young Thornless CRUB 2003.001 | NCGR Louisiana | 8-SSR |                               |
Supplemental Table 2. R code used to construct phylogenetic trees in the package “Poppr” (Kamvar et al., 2014, 2015) using a Bruvo’s infinite allele, allele-sharing model (Bruvo et al., 2004; Metzger et al., 2016).

# This block of code calls the packages `poppr` and `ape`
library(poppr)
library(ape)

# This block of code imports the data from a text file. Data is imported from a .txt file containing
# the accessions, ploidy, and markers arranged in columns. Allele calls for each marker is
# formatted such that each observed allele is separated by a colon ":" (e.g., 143:146). If SNP data
# is in use, recode the SNPs as number 1-5 where one number indicates a deletion and the
# remaining numbers indicate the nucleotide. Missing data is coded as "NA".

Mydat_table = read.table(choose.files(), header = TRUE, stringsAsFactors = FALSE)
Accession_Names = as.character(Mydat_table[,1]) #Column 1 of input file
Ploidy = as.integer(Mydat_table[,2]) #Column 2 of input file
Allele_Dat = Mydat_table[3:ncol(Mydat_table)] #Column 3 to end of input file
Mydat_genind = df2genind(Allele_Dat, sep = ";", ind.names = Accession_Names, ploidy = Ploidy, NA.char = "NA")
Mydat_genind_recoded = recode_polyploids(Mydat_genind, addzero = TRUE)

# This block of code performs the phylogenetic analysis for a Bruvo’s infinite allele, allele
# sharing model

ssr_replen = rep(0.0001, length(Mydat_genind@all.names))
Mytree = bruvo.boot(Mydat_genind_recoded, replen = ssr_replen, sample = 2000, cutoff = 70, tree = "njs", showtree = FALSE, add = FALSE, loss = FALSE)

# This block of code interactively performs K-means clustering

Mydat_clusters = find.clusters(Mydat_genind, scale = FALSE)

# This block of code plots the tree produced with K-means clusters overlayed as different colors
# for each group. See plot.phylo help for all plotting options

clustcolor = rainbow(length(unique(Mydat_clusters$grp)))
plot.phylo(Mytree, cex = 0.5, edge.width = 0.5, font = 2, label.offset = 0.00125, show.node.label = FALSE, type = "phylogram", tip.color = clustcolor[Mydat_clusters$grp], no.margin = TRUE)
nodelabels(Mytree$node.label, adj = c(1.2, -0.4), frame = "n", cex = 0.25)
legend(locator(), legend = c(1:length(clustcolor)), fill = clustcolor, cex = 0.5, bty = "n", ncol = 4, title = "K-means Clusters")
add.scale.bar(lwd = 0.5, cex = 0.5, font = 2, ask = TRUE)