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The Importance of Being 'Boysen': Examining Genotypic Variation With SSR Markers

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Abstract

Multiple variants of 'Boysen' have been horticulturally recognized since the cultivar was introduced in 1938. An improved 8-SSR DNA fingerprinting set was applied to 'Boysen' and 'Logan' variants and their putative parents obtained from private growers and commercial nurseries. A 'Boysen' genotype likely to be from the original clone was identified. 'Boysen' appears to have resulted from 'Logan' x 'Austin Mayes'. 'Lucretia' is not a parent. Continuing work will establish pedigree or relational links for wild and cultivated blackberries.

'Boysen' blackberry was introduced in 1935 in California and became a standard in the industry through the 1900s (Jennings, 1988). This berry has strong public recognition, although growers have shifted away from large-scale production of this cultivar. It is an important founding cultivar for the Pacific Northwest blackberry industry (Hall and Funt, 2017).

The origin of 'Boysen' remains obscure. It was developed by Rudolph Boysen, a hobbyist plant breeder who reportedly crossed a raspberry, a blackberry, and 'Logan' to create 'Boysen' (Stellar, 1937). Jennings (1988) later hypothesized that 'Boysen' resulted from a cross between 'Logan' and a trailing blackberry such as 'Lucretia' or 'Austin Mayes' (Jennings, 1981; 1988). He gave plants of his new hybrid to D.W. Coolidge of Coolidge Rare Plant Gardens (Pasadena, CA). Coolidge then informed Dr. George Darrow, USDA Bureau of Plant Industry (now the USDA-Agricultural Research Service) senior pomologist and berry breeder in Beltsville, MD, of the existence of this exciting cultivar. Darrow visited California to meet Boysen and identify the original plants. Walter Knott trialed these plants and found the fruit to be large and flavorful. He began selling plants in 1935 prior to Darrow's official release of the cultivar in June 1938 (Stellar, 1937; Wood et al. 1999).

Over the years, variants of 'Boysen' were selected internationally. These variants included the New Zealand selection 'Riwaka Choice' and an Oregon selection named 'Boysen 43' that became an industry standard in the 1990s (J. DeFrancesco, personal communication 2018).

The USDA-ARS National Clonal Germplasm Repository (NCGR) *Rubus* germplasm includes heritage cultivars, such as the 'Boysen' variants, along with crop wild relatives. DNA-based fingerprinting of this valuable germplasm complements morphological characterization. Simple sequence repeat (SSR) markers are used to establish genetic

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identify and familial relationships and assess germplasm diversity (Gökirmak et al., 2009; Bassil et al., 2015). Recently, an 8-SSR fingerprinting set was developed for blackberry (Zurn et al., 2018) that could clarify the parentage of 'Boysen' and its relationship with other heritage hybrid berries.

The objectives of this study were to assess variation in 'Boysen' clones from different sources and examine putative pedigree relationships between other early hybrid berries using SSR markers.

Materials and Methods

DNA extraction

Young leaf tissue was collected from 'Boysen', 'Logan', and associated cultivars from the NCGR *Rubus* collection, three independent private grower sites (PF1, PF2, and PF3), the USDA-ARS National Clean Plant Network (NCPN), the USDA-ARS, Horticultural Crops Research Unit (Corvallis, OR) (ORUS) breeding program, and four commercial nurseries (N1, N2, N3, and N4; Table 1). Specimens for 'Nectar Boysenberry' and 'Austin Mayes' were acquired from N2 in 2012. New 'Austin Dewberry' and 'Nectar Boysenberry' plants were acquired from N2 in 2018 due to inconsistencies. DNA was extracted using a modified Puregene protocol (Gentra Systems Inc. Minneapolis, MN, U.S.A.; Gilmore, 2011).

Genotyping and data analysis

All samples were genotyped with an 8-SSR multiplexed assay developed for blackberries (Zurn et al., 2018). DNA was PCR amplified using the Type-it® Microsatellite PCR Kit (Qiagen, Inc., Valencia, CA, U.S.A.) as described by Zurn et al., 2018. PCR products were separated by capillary electrophoresis with a Beckman CEQ 8000 (Beckman Coulter, Inc.). Electropherogram visualization and allele calling was performed with the CEQ 8000 software fragment analysis module.

An unrooted neighbor-joining phylogeny was constructed using the allele sharing model of R v 4.3.3 Bruvo's infinite allele, using the package 'Poppr' v 2.6.1 (Bruvo et al., 2004; Paradis et al., 2004; Kamvar et al., 2014; R Core Team, 2015) as described in Zurn et al. (2018). Allele compositions of samples were compared to determine if historical pedigree reports could be supported.

Results and Discussion

Misidentified hybrid berries abound

The eight SSRs amplified in all 29 individuals and generated 16 unique genotypes. Many hybrid berries with different names had matching fingerprints (Fig. 1). 'Young Thornless' from PF3, which originated in a private garden in Washington State, had fingerprints that matched that of 'Thornless Logan' and is likely misidentified. Specimens for 'Nectar Boysenberry' and 'Austin Mayes' from N2 in 2012 had matching genotypes (Fig. 1), and were hexaploid (Hummer et al., 2015) when expected to be octoploid and septaploid, respectively (Jennings, 1988). Their identity is unknown at this time. 'Austin Dewberry' from N2 obtained in 2018 matched a 'Boysen' genotype and is probably mislabeled.

Five 'Boysen' genotypes

Five genotypes were observed for "'Boysen'-like" accessions (Fig. 1). One 'Boysen' genotype was observed for the 'Boysen' accessions from PF1 and PF2, the 'Thornless Boysen' from N1, and the 'Austin Dewberry' from N2 obtained in 2018. The second genotype was exhibited by 'Riwaka Choice', the three plants from the NCPN, and 'Boysen ORUS'. 'Boysen 43', 'Boysenberry' from the NCGR, and 'Boysen' from N3 each had unique genotypes (Fig. 1).

The identical allele compositions, Californian origin, and history of the 'Boysen' plants from PF1 and PF2 suggest that these likely represent the original heritage 'Boysen' genotype. The PF1 grower is the granddaughter of Rudolph Boysen, and the plant originated from the Napa Valley Creek stand originally established by Rudolph Boysen (Jeanette Fitzgerald, Personal Communication; Court-

Table 1. 'Boysen' and associated heritage cultivars examined with an 8-SSR fingerprinting set. PI and local numbers are listed for accessions acquired from the USDA NCGR. Thorniness is indicated with 'Y' and thornlessness with a 'T'.

Name as received	Thorniness (T/Y)	Ploidy	Source	Local number	PI number	Pedigree/origin based on name as received
Austin_Dewberry_N2_18	Y	2*n*=8*x*=56	Nursery 2	n/a	n/a	*R. baileyanus x R. argutus*
Austin_Mayes_N2_12	Y	2*n*=8*x*=56	Nursery 2	CRUB 2653.001	PI 672692	*R. baileyanus x R. argutus*
Austin_Thornless	T	2*n*=8*x*=56	NCGR	CRUB 357.001	PI 553292	Thornless seedling/sport of 'Austin Mayes'
Bauers_Thornless Logan	T	2*n*=6*x*=42	NCGR	CRUB 81.001	PI 553258	Thornless sport of 'Logan'
Boysen_43	Y	2*n*=7*x*=49	NCGR	CRUB 1108.001	PI 553341	Variant of 'Boysen'
Boysen_ORUS	Y	2*n*=7*x*=49	USDA ARS Lab 1	n/a	n/a	'Logan' x unknown trailing blackberry
Boysen_NCPN1	Y	2*n*=7*x*=49	USDA ARS Lab 2	n/a	n/a	'Logan' x unknown trailing blackberry
Boysen_NCPN2	Y	2*n*=7*x*=49	USDA ARS Lab 2	n/a	n/a	'Logan' x unknown trailing blackberry
Boysen_NCPN3	Y	2*n*=7*x*=49	USDA ARS Lab 2	n/a	n/a	'Logan' x unknown trailing blackberry
Boysen_PF1	Y	2*n*=7*x*=49	CA Private Farm 1	n/a	n/a	'Logan' x unknown trailing blackberry
Boysenberry	Y	2*n*=7*x*=49	NCGR	CRUB 1025.001	PI 553336	'Logan' x unknown trailing blackberry
Boysen_N3	Y	2*n*=7*x*=49	Nursery 3	CRUB 2894.001	PI 687271	'Logan' x unknown trailing blackberry
Lincoln_Logan	T	2*n*=6*x*=42	NCGR	CRUB 737.001	PI 553314	TC derived, thornless 'Logan'
Logan_BE1132	T	2*n*=6*x*=42	NCGR	CRUB 2535.001	PI 687013	'Aughinbaugh' x 'Red Antwerp'
Loganberry_seedling1	Y	2*n*=6*x*=42	NCGR	CRUB 2657.002	PI 672696	'Logan' x unknown
Loganberry_seedling2	Y	2*n*=6*x*=42	NCGR	CRUB 2657.001	PI 672696	'Logan' x unknown
Lucretia	Y	2*n*=7*x*=49	NCGR	CRUB 74.001	PI 553253	*R. baileyanus x R. argutus*
Nectar_Boysenberry_N2_12	Y	2*n*=7*x*=49	Nursery 2	CRUB 2654.001	PI 672693	'Young' x unknown; 'Boysen' variant
Nectar_Boysenberry_N2_18	Y	2*n*=7*x*=49	Nursery 2	n/a	n/a	'Young' x unknown; 'Boysen' variant
Nectarberry	Y	2*n*=7*x*=49	NCGR	CRUB 1864.001	PI 618436	'Young' x unknown; 'Boysen' variant
Phenomenal	Y	2*n*=6*x*=42	NCGR	CRUB 1817.001	PI 618478	'Aughinbaugh' x 'Cuthbert'
Riwaka_Choice	Y	2*n*=7*x*=49	NCGR	CRUB 2207.001	PI 638239	'Boysen' selection in New Zealand
Thornless_Boysen_N1_18	T	2*n*=7*x*=49	Nursery 1	CRUB 2892.001	PI 687270	Thornless sport of 'Boysen'
Thornless_Logan_N1_18	T	2*n*=6*x*=42	Nursery 1	n/a	n/a	Thornless sport of 'Logan'
Thornless_Logan_N3	T	2*n*=6*x*=42	Nursery 3	n/a	n/a	Thornless sport of 'Logan'
Logan_N4	T	2*n*=6*x*=42	Nursery 4	n/a	n/a	Thornless sport of 'Logan'
Young	Y	2*n*=7*x*=49	NCGR	CRUB 131.001	PI 553266	'Phenomenal' x 'Austin Mayes'
Young_Thornless_PF3	T	2*n*=6*x*=42	NCGR	CRUB 2003.001	PI 618391	Thornless sport of 'Young'
Boysen_PF2	Y	2*n*=7*x*=49	CA Private Farm 2	CRUB 2809.001	PI 684800	'Logan' x unknown trailing blackberry

ney, 2016). The 'Boysen' plant from the PF2 grower was originally purchased from Knott's Berry Farm about 70 years ago and was grown in Buena Vista, California.

'Thornless Boysen' from N1 is likely a clonal thornless variant of the heritage genotype because these samples are indistinguishable by the 8-SSR assay. Clonal variants are rarely distinguished by SSRs (Bassil and Postman, 2010; Cretazzo et al., 2010). Other 'Boysen' variants differed from the heritage genotype by multiple alleles for at least five of the eight SSR loci. The level of variation observed is typical of a parent-offspring or half-sibling relationship.

Parentage of 'Boysen'

Historically, 'Boysen' was hypothesized by Jennings to be a cross between 'Logan' and an unknown trailing blackberry similar to 'Lucretia' (1988) or 'Austin Mayes' (1981, 1988). DNA fingerprinting uncovered a close relationship between 'Boysen', 'Young' and 'Austin Thornless' (Nybom and Hall, 1991). Because we were unable to acquire a reliable 'Austin Mayes' genotype, 'Austin Thornless', a thornless sport of 'Austin Mayes' (Butterfield 1928), was used as its proxy in allele comparisons. Two 'Logan' genotypes were observed; one is exhibited by accession Logan-BE1132 (thornless) from Bologna, Italy, and the other is seen in six other thornless variants of 'Logan'. Each 'Logan' genotype was assessed for parentage of 'Boysen' using either 'Lucretia' or 'Austin Thornless' as the pollen parent. With Logan-BE1132 as the maternal parent and 'Lucretia' as the pollen parent, the hypothesized original 'Boysen' genotype had unique alleles not found in either parent at six SSR loci. When 'Austin Thornless' was the pollen parent, unique alleles were only seen at two loci. The original 'Boysen' genotype also had unique alleles at six loci when assessing the 'Thornless Logan'

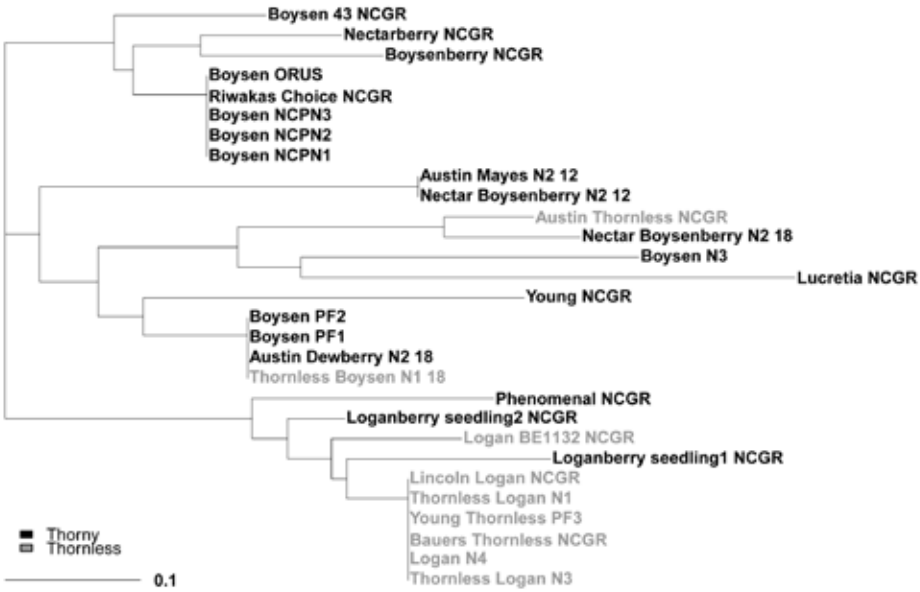


Figure 1. Neighbor joining cluster analysis of 29 hybrid berry accessions based on Bruvo's distance from an 8-SSR blackberry fingerprinting set. Multiple 'Boysen' genotypes, 'Logan' genotypes, and hybrid berry cultivars from private farms (PF) and nurseries (N), some of which are likely mislabeled, are illustrated. ORUS refers to the Oregon, USDA-ARS Horticultural Crop Research program in Corvallis, OR. NCPN is the National Clean Plant Network in Corvallis, OR. Hybrid berry plants were obtained from four nurseries (N1, N2, N3 and N4), three private farms (PF1, PF2, and PF3) and the USDA-ARS National Clonal Germplasm Repository collection in Corvallis, OR (NCGR). Thorniness is indicated by black font while lack of thorns is indicated by grey font. The scale bar measures Bruvo's genetic distance along each branch.

x 'Lucretia' cross. However, 'Thornless Logan' x 'Austin Thornless' could have resulted in the hypothesized original 'Boysen' genotype. We can eliminate 'Lucretia' as a possible parent and infer that the pollen parent was 'Austin Mayes' or a close relative. This is supported by attempts in New Zealand to re-create 'Boysen' from crossing 'Austin Thornless' with 'Lincoln Logan' (Hall et al., 2002) and previous DNA fingerprinting indicating a close relationship between 'Austin Thornless' and 'Boysen' (Nybom and Hall, 1991). The six accessions with the 'Thornless Logan' genotype are likely true thornless variants of the original 'Logan' cultivar due to their consistent genotype and the plausibility that a cultivar with this genotype was the maternal parent of 'Boysen'. 'Lincoln Logan' is a genetically thornless cultivar derived using tissue culture from 'Thornless Logan' (Hall et al., 1986). 'Thornless Logan' may be an abbreviated name adopted by the industry for 'Bauer Thornless Loganberry', explaining the identical fingerprints of these three accessions. The original 'Logan' cultivar was thorny, but we were unable to acquire any 'Logan' types with prickles after searching local nurseries and national breeding programs. This genotype may have been lost in favor of the thornless type. Logan-BE1132, as well as the two Loganberry seedlings 1 and 2 are possibly 'Logan' offspring, as they share alleles with 'Logan' at all loci but have unique alleles, putatively from the other parent, at multiple loci.

Alternatively, the 'Lucretia', 'Austin Mayes', and 'Logan' plants evaluated may not represent historical specimens of these cultivars. Mutations and propagation errors could have led to different genotypes. As seen in the five 'Boysen' genotypes, individual plants with widely differing genotypes can be acquired under the same name. A search of US berry breeding programs and private nurseries yielded no other reliable sources of 'Logan', 'Lucretia', or 'Austin Mayes'. Other global sources of these heritage cultivars will be investigated.

Relationships of 'Boysen' "clones"

'Nectarberry', 'Nectar Boysenberry', 'Riwaka's Choice', and 'Boysen 43' differed from the putative 'Boysen' heritage genotype at five to seven SSRs and are more likely seedlings of the heritage genotype or related by pedigree than clonal variants. At this time parentage inference is not possible in polyploids and we rely on comparing allele composition between putative parents and offspring to confirm such relationships.

In conclusion, genotyping heritage hybrid blackberry cultivars at eight SSR loci exemplifies the variability in accessions with similar names. Genetic characterization of germplasm is necessary to curate true-to-type specimens. Breeders or buyers may need to confirm the authenticity of desired cultivars because of misidentified plants in the trade. The hypothesized original 'Boysen' genotype was likely derived from a 'Logan' x 'Austin Mayes' or similar cross. Further research is planned to contrast morphological and fruit quality differences for 'Boysen' variants.

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