



## Genetic relatedness among eastern North American blackberry cultivars based on pedigree analysis

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### Summary

Blackberry genetic relationships (*Rubus* subgenus *Rubus* Watson) have not been thoroughly studied. The University of Arkansas maintains one of the largest blackberry-breeding programs in the world and thus, in-depth knowledge of the cultivars released from the program and others instrumental in their lineages can aid in breeding endeavors. Pedigrees of 32 cultivars were traced to their founding clones. Genetic contribution (GC), maximum potential similarity (MPS), inbreeding coefficient, and coefficients of relationship (CR) were calculated for all genotypes. Nineteen founding clones contributed to the 32 cultivars, ranging from <1% to ~19%. The top three founding clones for mean GC conferred nearly 50% to the 32 cultivars in this study, suggesting a somewhat narrow genetic base. Calculations for MPS ranged from complete similarity (1.00) to 0.00. Individual inbreeding coefficients ranged from 0.00 to 0.25. The coefficient of relationship was used as a method of determining genetic relatedness and ranged from 0.00 to 0.74. University of Arkansas cultivars had the highest MPS and CR in the study. This may be attributed to more generations of hybridization than the other cultivars. The MPS provided some comparable results to those of RFLP and RAPD molecular studies, but CR did not. The rather narrow genetic base of eastern North American blackberry cultivars does not create an imminent, significant concern for loss of diversity. However, more diverse germplasm should be infused for continuation of heterogeneity.

*Abbreviations:* APF: Arkansas primocane fruiting selection; CR: coefficient of relationship; GC: genetic contribution; MPS: maximum potential similarity

### Introduction

Blackberry (*Rubus* subgenus *Rubus* Watson) breeding has been practiced for well over 150 years, with the first cultivars from a breeding program released in 1880 (Hall, 1990). In 1909, the first public blackberry-breeding program was started in Texas (Moore, 1984). The University of Arkansas blackberry-breeding program was initiated in 1964 and has since released 13 cultivars, of which 10 are patented and/or trademarked. It is the largest blackberry-breeding program in the United States that utilizes strictly eastern North American blackberry germplasm. Some of the major objectives of this program are to develop superior

genotypes that contain the following traits: improved thornless character, erect canes, fruit firmness, large fruit size, high yield, and, recently, primocane fruiting (Clark, 1999).

The Arkansas breeding program, as well as several other programs, has relied heavily on genotypes derived from *Rubus allegheniensis* Porter and *R. argutus* Link, the base species for eastern North American blackberries. Many of the genotypes used in the early years of the program were from the Texas A&M breeding program which was mature and established at the time. Several of the other programs that have released blackberry cultivars no longer maintain breeding programs or have greatly diminished their output. The

only other public eastern U.S. program to release a blackberry since 1988, aside from the University of Arkansas, is the University of Maryland which released 'Chesapeake' in 2002.

Breeding for new blackberry genotypes has potentially led to a narrowing of the genetic diversity due to the aforementioned reliance on only a few founding clones. The lack of genetic variability in any crop can potentially lead to the surfacing of deleterious recessive traits. However, the current practice of intensive crossing among selections within the Arkansas blackberry-breeding program has led to important discoveries with economic potential within the blackberry genome, such as thornless canes and primocane fruiting. Yet, inbreeding also limits the amount of progress that can be gained versus that of an outcrossing population (Hall, 1990).

Pedigree analysis is a well-established method to estimate inbreeding and genetic diversity in small fruit crops (Dale et al., 1993; Degani et al., 2001; Hancock & Siefker, 1982; Sjulín & Dale, 1987). Yet, it has not been used extensively for blackberries, other than a recent study by Stafne et al. (2003). Blackberry is a highly heterozygous plant and many current cultivars are tetraploid. Because of the varied reproductive strategies (sexual, facultatively apomictic, and obligately apomictic; Hall, 1990), cytological conditions (auto- and allo-polyploidy), and inheritance strategies (disomic and tetrasomic) (Lopez-Medina et al., 2000) of polyploid blackberries, it is potentially difficult to accurately portray genetic contribution through pedigree records. However, since eastern North American blackberries for the most part are not apomictic (Hall, 1990), sexual recombination can be assumed. An equal segregation of genes from parent to progeny will also be assumed in this study because allopolyploidy is more prevalent in *Eubatus* (the blackberry subgenus) than autopolyploidy (Ourecky, 1975), thus leading to bivalent chromosome pairing. In another study of a polyploid, heterozygous crop, Lima et al. (2002) used pedigree analysis to characterize sugarcane relations successfully. Therefore, pedigree analysis can provide a reliable basis for comparison of genotypes.

The objectives of this study were to estimate the level of inbreeding and determine genetic similarity among a range of eastern North American blackberry cultivars using inbreeding coefficients, coefficients of relationship (CR), and maximum potential similarity (MPS) from the genetic contribution (GC) of the founding clones for the assessment of genetic diversity. The

results were also compared to previously published molecular marker studies to assess viability of the pedigree analysis.

## Materials and methods

Thirty-two eastern North American blackberry cultivars developed east of the Rocky Mountains were included in this study because, (1) they are, have been, or may be potentially important to the industry (2) they are representatives from a variety of eastern North American states and (3) all have known lineages that could be traced to founding clones (Table 1). Many other cultivars could have been included, but some are full sibs to other cultivars used in this study or have questionable information documenting their parentage. Therefore, only cultivars with reliable background information were used. The parental information for these cultivars and intermediate ancestors was entered into a specialized program developed in Microsoft<sup>®</sup> Access 2000<sup>™</sup>, PediTrack (Stafne & Clark, 2004), which traced all pedigrees back to their founding clones. Parentage information was gained from numerous sources, including Darrow (1937), Hedrick (1925), C. Finn (personal communication), and records from the National Clonal Germplasm Repository (NCGR) in Corvallis, Ore. If possible, the original published pedigree was used. For University of Arkansas blackberry pedigrees, internal breeding program parental records were referenced.

Genetic contribution (GC) was calculated as  $GC = \sum (1/2)_{1...x}^n$ , where n is equal to the number of generations between the founding clone and the cultivar and x is the number of generational pathways between the founding clone and the cultivar (Dale et al., 1993). Open-pollinated genotypes were considered to have an unknown male parent.

Maximum potential similarity (MPS) takes into account pairwise comparisons between cultivars for each founding clone they share. Unshared founding clones were ignored in this comparison, as were unknown (OP) clones. When the shared founding clones were compared, the lesser of the two values was selected and totaled for all shared founding clones. This total was attributed the name MPS because it determined how much potential genetic contribution one genotype could share with another.

Inbreeding coefficients (Wright, 1922) and coefficients of relationship (sometimes referred to as covariance coefficient or theoretical correlation)

Table 1. Parentage and inbreeding coefficient (F) of 32 eastern North American blackberry cultivars

Genotype	Parentage	Program	Year of release	F
Apache	(SIUS 68-6-15 × Comanche) × Navaho	University of Arkansas	1998	0.17
Arapaho	(Ark.-550 × Cherokee) × Ark.-883	University of Arkansas	1993	0.11
Brazos	Lawton × Nessberry	Texas A&M University	1959	0.00
Brewer	<i>R. pergratus</i> × <i>R. frondosus</i>	Wild Selection (New Jersey)	1920	0.00
Cherokee	Darrow × Brazos	University of Arkansas	1974	0.06
Chesapeake	Crisfield × Shawnee	University of Maryland	2002	0.00
Chester	SIUS 47 × Thornfree	University of Illinois	1985	0.16
Cheyenne	Darrow × Brazos	University of Arkansas	1976	0.06
Chickasaw	(Comanche × Ark.-516) × Ark.-1246	University of Arkansas	1998	0.22
Choctaw	(Darrow × Brazos) × Rosborough	University of Arkansas	1988	0.14
Comanche	Darrow × Brazos	University of Arkansas	1974	0.06
Darrow	(Eldorado × Brewer) × Hedrick	Cornell University	1958	0.25
Early June	(Himalaya × Eldorado) × NC 36	University of Georgia	1959	0.00
Eldorado	<i>R. allegheniensis</i> × <i>R. argutus</i>	Wild Selection (Ohio)	1880	0.00
Flint	Brainerd × Eldorado	University of Georgia	1957	0.00
Gem	Flint × Early June	University of Georgia	1967	0.11
Illini Hardy	NY 95 × Chester	University of Illinois	1988	0.00
Kiowa	(Ark.-586 × Comanche) × (Ark.-628 × Rosborough)	University of Arkansas	1996	0.13
Lawton	<i>R. allegheniensis</i> × <i>R. frondosus</i>	Wild Selection (New York)	1848	0.00
Mayes	<i>R. baileyanus</i> × <i>R. argutus</i>	Wild Selection (Texas)	1880	0.00
Mersereau	<i>R. argutus</i> × <i>R. frondosus</i>	Wild Selection (New York)	1890	0.00
Navaho	(Thornfree × Brazos) × (Ark.-550 × Cherokee)	University of Arkansas	1988	0.13
Ouachita	Navaho × Ark.-1506	University of Arkansas	2003	0.07
Prime-Jan <sup>TM</sup>	Ark.-1836 × Arapaho	University of Arkansas	2004	0.12
Prime-Jim <sup>TM</sup>	Arapaho × Ark.-830	University of Arkansas	2004	0.18
Ranger	Eldorado × Dew-black	University of Maryland	1964	0.00
Rosborough	F <sub>2</sub> (Brainerd × Brazos) × Brazos	Texas A&M University	1977	0.00
Shawnee	Cherokee × (Thornfree × Brazos)	University of Arkansas	1985	0.16
Smoothstem	[(Merton × (Merton × Eldorado))] O.P.	USDA-ARS (Maryland)	1966	0.00
Thornfree	(Brainerd × Merton) × (Merton × Eldorado)	USDA-ARS (Maryland)	1966	0.16
Triple Crown	SIUS 47 × (Darrow × Brazos)	USDA-ARS (Maryland)	1996	0.12
Williams	Himalaya × Taylor	USDA-ARS/NC State	1962	0.00

(Barr, 1983; Falconer & Mackay, 1996) were derived in the SAS INBREED procedure (version 8.0) with the COVAR, IND, and MATRIX options. Coefficient of relationship is the covariance between individual X and individual Y, where  $f_{XY}$  indicates the coancestry between X and Y. Therefore, the covariance  $Cov(X, Y)$  is equal to  $2f_{XY}$ , as defined by Barr (1983). It is essentially the correlation of the breeding values between two individuals and is a representation of the correlation if all phenotypic variance observed was genetically additive. Two main assumptions were

followed: bivalent meiotic chromosome pairing and open-pollinated genotypes were considered to have an unknown male parent.

Construction of dendrograms was derived from the application of the unweighted pair-groups method average (UPGMA) to the similarity matrices in the TREE program from the Numerical Taxonomy and Multivariate Analysis System for PC (NTSYS-pc, version 2.1) (Rohlf, 2000). A cophenetic correlation (product-moment correlation) was used to determine the goodness-of-fit of the UPGMA cluster

dendrograms to the MPS and CR similarity matrices using ultrametric distances with the Mantel test (Mantel, 1967) in the COPH and MXCOMP programs, as well as a matrix correlation between MPS and CR using the same test.

## Results

A total of 19 founding clones are known to have contributed to the 32 blackberry genotypes in this study. *Rubus allegheniensis*, *R. argutus*, and *R. frondosus* Bigel had the greatest frequencies and were present in at least 22 of the 32 cultivars (Table 2). *R. allegheniensis* had the highest mean GC of 18.56%, indicating that according to pedigree records, it comprised nearly 20% of the total genetic makeup over all of the 32 cultivars in this study. Conversely, *R. occidentalis* L., a black raspberry species, contributed only 0.05%, having been in only 'Prime-Jan'<sup>TM</sup>. Eleven of the 19 founding clones had a frequency of 12 or more and six had a frequency of only one (Table 2). Overall, the top three founding clones for mean GC conferred nearly 50% to the 32

Table 2. Frequency of occurrence and mean genetic contribution (GC) of founding clones in 32 eastern North American blackberry cultivars

Clone no.	Clone	Frequency	Mean GC (%)
1	<i>Rubus allegheniensis</i> Porter	29	18.56
2	<i>Rubus argutus</i> Link.	28	15.53
3	<i>Rubus frondosus</i> Bigel.	22	14.71
4	<i>Rubus procerus</i> Muell.	20	6.56
5	<i>Rubus strigosus</i> Michx.	17	5.91
6	<i>Rubus rubrisetus</i> Rydb.	17	5.86
7	<i>Rubus pergratus</i> Blanch.	18	5.73
8	Unknown (OP)	12	5.34
9	<i>Rubus thyriger</i> Banning & Focke	15	3.89
10	<i>Rubus ulmifolius</i> var. <i>inermis</i> Focke	15	3.89
11	Georgia Mammoth	18	3.82
12	NC 36	2	2.34
13	Dew-black	1	1.56
14	NY 95	1	1.56
15	<i>Rubus baileyanus</i> Britt.	1	1.56
16	<i>Rubus cuneifolius</i> Pursh	1	1.56
17	Well's Beauty	3	1.17
18	SIUS 68-1-8	1	0.39
19	<i>Rubus occidentalis</i> L.	1	0.05

cultivars in this study, suggesting a somewhat narrow genetic base.

Inbreeding coefficients for the 32 cultivars ranged from a low of 0.00 for several cultivars to 0.25 for 'Darrow' (Table 1). Since the coefficient of inbreeding is an assessment of the probability that two alleles at any particular locus are identical by descent, any value greater than zero indicates a presence of inbreeding (Falconer & Mackay, 1996). Fourteen of the 32 cultivars in this study have inbreeding coefficients greater than 0.10 (Table 1), nine of which are from the University of Arkansas program and the remainder are 'Chester', 'Darrow', 'Gem', 'Thornfree', and 'Triple Crown'. Another 14 of the 32 cultivars had inbreeding coefficients of 0.00 indicating a lack of inbreeding in those individuals (Table 1).

Maximum potential similarity (MPS) was used to calculate the similarity among cultivars. The MPS ranged from a high of 1.00 (complete similarity) to 0.00 (complete dissimilarity) (Table 3). Due to the nature of the MPS calculation, clones with identical parentage were not discernible. Overall mean MPS for all cultivars was 0.39 and ranged from 0.14 for 'Mayes' to 0.54 for 'Shawnee'. The Arkansas cultivars averaged a MPS of 0.50, whereas non-Arkansas bred cultivars had a MPS of 0.32. When the cultivars are divided into geographic regions, Arkansas still has the highest average MPS compared to Texas (0.32), New York/New Jersey (0.31), Maryland/USDA-ARS (0.33), Illinois (0.29), Georgia (0.32), and Ohio (0.32).

The coefficient of relationship (CR) was also used as a measure of relatedness and intermediate ancestors are taken into account when the CR is calculated. However, in the calculation of CR it is presumed that the ancestors are not closely related, which may or may not be true in the case of *Rubus*. Therefore, even though the calculation of CR may be informative it also may not represent the full picture of relationship between the genotypes in this study. Values for CR ranged from 0.00 to 0.74 for 'Thornfree'/'Chester Thornless' (Table 3).

Matrices of both MPS and CR were subjected to the Mantel (1967) test for matrix correlation. The test resulted in a correlation  $r = 0.84$ , suggesting that the MPS and CR techniques offer similar overall assessment of the data, though specific clustering results are not identical. This result suggests that pedigree analysis based on founding clones (MPS) is a valid measurement of genetic relatedness when compared with CR.

Cluster analysis was performed for both MPS and CR similarity matrices using the UPGMA method

Table 3. Coefficients of relationship (CR) and maximum similarity coefficients (MPS) for 32 eastern North American blackberry cultivars

	Apache	Prime-Jan	Prime-Jim	Arapaho	Cherokee	Cheyenne	Chickasaw	Choctaw	Comanche	Kiowa	Navaho	Ouachita	Shawnee	Brazos	Rosborough	Chesapeake
Apache	1.00	0.31 <sup>z</sup>	0.37	0.41	0.45	0.40	0.39	0.31	0.51	0.32	0.74	0.45	0.42	0.36	0.21 <sup>z</sup>	0.21
Prime-Jan	0.70	1.00	0.48	0.68	0.40	0.32	0.27	0.31	0.32	0.28	0.36	0.23	0.35	0.40	0.31	0.18
Prime-Jim	0.78	0.71 <sup>y</sup>	1.00	0.74	0.42	0.37	0.32	0.28	0.37	0.27	0.40	0.26	0.38	0.35	0.20	0.19
Arapaho	0.75	0.73	0.82	1.00	0.48	0.37	0.31	0.30	0.37	0.27	0.50	0.31	0.41	0.40	0.23	0.20
Cherokee	0.68	0.63	0.63	0.66	1.00	0.63	0.49	0.45	0.63	0.35	0.47	0.31	0.69	0.56	0.28	0.35
Cheyenne	0.68	0.63	0.63	0.66	1.00	0.78	0.49	0.45	0.63	0.35	0.36	0.26	0.47	0.56	0.28	0.24
Chickasaw	0.77	0.79	0.72	0.73	0.78	1.00	0.34	0.34	0.65	0.34	0.32	0.24	0.39	0.37	0.20	0.19
Choctaw	0.69	0.66	0.62	0.65	0.75	0.75	1.00	0.69	0.45	0.39	0.30	0.21	0.39	0.53	0.64	0.19
Comanche	0.68	0.63	0.63	0.66	1.00	1.00	0.78	0.75	1.00	0.46	0.36	0.28	0.47	0.56	0.28	0.24
Kiowa	0.71	0.83	0.68	0.71	0.63	0.63	0.77	0.78	0.63	1.00	0.30	0.22	0.42	0.46	0.44	0.21
Navaho	0.90	0.71	0.74	0.76	0.64	0.64	0.70	0.70	0.64	0.75	1.00	0.63	0.44	0.41	0.24	0.22
Ouachita	0.62	0.56	0.61	0.58	0.45	0.45	0.54	0.50	0.45	0.58	0.62	1.00	0.30	0.27	0.16	0.15
Shawnee	0.84	0.72	0.76	0.77	0.81	0.81	0.80	0.81	0.81	0.77	0.83	0.55	1.00	0.54	0.30	0.58
Brazos	0.50	0.47	0.45	0.49	0.75	0.75	0.55	0.63	0.75	0.52	0.51	0.35	0.66	1.00	0.50	0.27
Rosborough	0.56	0.56	0.50	0.54	0.63	0.63	0.56	0.88	0.63	0.70	0.59	0.40	0.69	0.75	1.00	0.15
Chesapeake	0.48	0.56	0.48	0.48	0.41	0.41	0.48	0.44	0.41	0.48	0.48	0.48	0.48	0.33	0.36 <sup>y</sup>	1.00
Smoothstem	0.31	0.18	0.23	0.21	0.13	0.13	0.19	0.13	0.13	0.21	0.28	0.24	0.25	0.06	0.06	0.16
Williams	0.34	0.33	0.44	0.38	0.38	0.38	0.34	0.38	0.38	0.30	0.30	0.22	0.34	0.25	0.31	0.17
Darrow	0.52	0.44	0.60	0.55	0.75	0.75	0.63	0.50	0.75	0.39	0.45	0.32	0.56	0.50	0.38	0.28
Gem	0.37	0.38	0.40	0.40	0.31	0.31	0.34	0.50	0.31	0.39	0.34	0.25	0.34	0.19	0.44	0.19
Eldorado	0.30	0.28	0.43	0.36	0.38	0.38	0.33	0.25	0.38	0.22	0.27	0.19	0.31	0.25	0.19	0.16
Thornfree	0.50	0.40	0.41	0.38	0.25	0.25	0.34	0.44	0.25	0.45	0.45	0.36	0.41	0.13	0.38	0.23
Ranger	0.30	0.28	0.41	0.36	0.38	0.38	0.33	0.25	0.38	0.22	0.27	0.19	0.31	0.25	0.19	0.16
Flint	0.38	0.38	0.46	0.41	0.38	0.38	0.36	0.50	0.38	0.39	0.34	0.25	0.38	0.25	0.44	0.19
Chester	0.50	0.40	0.41	0.38	0.25	0.25	0.34	0.31	0.25	0.40	0.45	0.36	0.41	0.13	0.25	0.23
Illini Hardy	0.45	0.34	0.35	0.35	0.22	0.22	0.31	0.28	0.22	0.35	0.44	0.37	0.41	0.11	0.17	0.27
Triple Crown	0.88	0.68	0.77	0.75	0.70	0.70	0.80	0.63	0.70	0.67	0.80	0.62	0.83	0.48	0.53	0.48
Brewer	0.22	0.16	0.19	0.19	0.38	0.38	0.30	0.25	0.38	0.17	0.19	0.13	0.25	0.25	0.19	0.13
Early June	0.27	0.30	0.28	0.27	0.25	0.25	0.27	0.31	0.25	0.27	0.25	0.22	0.25	0.13	0.25	0.17
Lawton	0.34	0.27	0.29	0.30	0.50	0.50	0.39	0.38	0.50	0.30	0.32	0.22	0.41	0.50	0.38	0.20
Mersereau	0.26	0.27	0.40	0.34	0.38	0.38	0.31	0.25	0.38	0.20	0.23	0.16	0.28	0.25	0.19	0.14
Mayes	0.11	0.14	0.27	0.20	0.13	0.13	0.13	0.06	0.13	0.05	0.09	0.06	0.09	0.00	0.00	0.05

(Continued on next page)

Table 3. (Continued)

	Smoothstem	Williams	Darrow	Gem	Eldorado	Thornfree	Ranger	Flint	Chester	Illini Hardy	Triple Crown	Brewer	Early June	Lawton	Mersereau	Mayes
Apache	0.14	0.10	0.43	0.12	0.27	0.40	0.13	0.17	0.40	0.20	0.40	0.18	0.08 <sup>z</sup>	0.25	0.13	0.06
Prime-Jan	0.05	0.11	0.24	0.10	0.19	0.20	0.09	0.14	0.19	0.09	0.25	0.11	0.06	0.23	0.13	0.07
Prime-Jim	0.09	0.13	0.39	0.13	0.30	0.31	0.15	0.18	0.30	0.15	0.33	0.15	0.08	0.22	0.20	0.13
Arapaho	0.08	0.11	0.34	0.11	0.25	0.27	0.13	0.15	0.26	0.13	0.31	0.14	0.07	0.25	0.17	0.10
Cherokee	0.04	0.09	0.69	0.12	0.31	0.08	0.16	0.16	0.23	0.12	0.43	0.31	0.08	0.38	0.19	0.06
Cheyenne	0.04	0.09	0.69	0.12	0.31	0.08	0.16	0.16	0.23	0.12	0.43	0.31	0.08	0.38	0.19	0.09
Chickasaw	0.07	0.09	0.60	0.12	0.29	0.21	0.14	0.16	0.29	0.14	0.39	0.26	0.08	0.27	0.16	0.06
Choctaw	0.02	0.16	0.38	0.16	0.19	0.11	0.09	0.22	0.16	0.08	0.31	0.19	0.09	0.31	0.13	0.03
Comanche	0.04	0.09	0.69	0.12	0.31	0.08	0.16	0.16	0.23	0.12	0.43	0.31	0.08	0.38	0.19	0.06
Kiowa	0.06	0.12	0.23	0.12	0.16	0.28	0.08	0.17	0.23	0.11	0.29	0.12	0.07	0.26	0.10	0.03
Navaho	0.10	0.10	0.32	0.11	0.22	0.40	0.11	0.15	0.33	0.16	0.35	0.14	0.07	0.25	0.11	0.04
Ouachita	0.08	0.07	0.24	0.08	0.16	0.29	0.08	0.11	0.25	0.12	0.26	0.10	0.05	0.17	0.06	0.03
Shawnee	0.09	0.10	0.41	0.12	0.25	0.34	0.13	0.16	0.31	0.16	0.39	0.19	0.07	0.33	0.14	0.05
Brazos	0.02	0.06	0.13	0.05	0.13	0.03	0.06	0.06	0.05	0.03	0.31	0.13	0.03	0.50	0.13	0.00
Rosborough	0.01	0.22	0.06	0.20	0.06	0.14	0.03	0.28	0.09	0.04	0.19	0.06	0.11	0.25	0.06	0.00
Chesapeake	0.04	0.05	0.20	0.06	0.13	0.17	0.06	0.08	0.16	0.08	0.20	0.09	0.04	0.16	0.07	0.02
Smoothstem	<b>1.00</b>	0.03	0.06	0.05	0.13	0.27	0.06	0.06	0.31	0.16	0.18	0.00	0.03	0.03	0.00	0.03
Williams	0.13	<b>1.00</b>	0.13	0.28	0.25	0.16	0.13	0.31	0.13	0.06	0.11	0.00	0.25	0.13	0.13	0.13
Darrow	0.13	0.50	<b>1.00</b>	0.19	0.50	0.13	0.25	0.25	0.41	0.20	0.55	0.50	0.13	0.25	0.25	0.13
Gem	0.13	0.38	0.38	<b>1.00</b>	0.38	0.18	0.19	0.61	0.16	0.08	0.14	0.00	0.61	0.09	0.09	0.09
Eldorado	0.13	0.50	0.50	0.38	<b>1.00</b>	0.25	0.50	0.50	0.31	0.16	0.31	0.00	0.25	0.25	0.25	0.25
Thornfree	0.41	0.38	0.25	0.50	0.25	<b>1.00</b>	0.13	0.25	0.74	0.37	0.41	0.00	0.11	0.06	0.06	0.06
Ranger	0.13	0.50	0.50	0.38	0.50	0.38	<b>1.00</b>	0.25	0.16	0.08	0.16	0.00	0.13	0.13	0.13	0.13
Flint	0.13	0.50	0.50	0.75	0.50	0.38	0.50	<b>1.00</b>	0.22	0.11	0.19	0.00	0.22	0.13	0.13	0.13
Chester	0.41	0.31	0.25	0.38	0.25	0.75	0.25	0.38	<b>1.00</b>	0.58	0.47	0.13	0.10	0.11	0.09	0.08
Illini Hardy	0.29	0.19	0.22	0.22	0.16	0.38	0.16	0.22	0.38	<b>1.00</b>	0.24	0.06	0.05	0.05	0.05	0.04
Triple Crown	0.29	0.38	0.59	0.39	0.34	0.48	0.34	0.41	0.48	0.45	<b>1.00</b>	0.05	0.09	0.24	0.14	0.07
Brewer	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.25	<b>1.00</b>	0.00	0.25	0.25	0.00
Early June	0.13	0.50	0.25	0.75	0.25	0.38	0.25	0.50	0.31	0.19	0.28	0.00	<b>1.00</b>	0.06	0.06	0.06
Lawton	0.06	0.25	0.50	0.19	0.50	0.13	0.25	0.25	0.13	0.11	0.36	0.50	0.13 <sup>y</sup>	<b>1.00</b>	0.25	0.00
Mersereau	0.06	0.25	0.50	0.19	0.50	0.13	0.25	0.25	0.13	0.11	0.30	0.50	0.13	0.50	<b>1.00</b>	0.25
Mayes	0.06	0.25	0.25	0.19	0.50	0.13	0.25	0.25	0.13	0.08	0.14	0.00	0.13	0.00	0.50	<b>1.00</b>

<sup>z</sup>Coefficients above the bold are coefficients of relationship.

<sup>y</sup>Coefficients below the bold are maximum similarity coefficients.

(data not shown). The MPS similarity gives a relative measure of relatedness, but because of its nature of calculation, it was not able to distinguish between ‘Cherokee’, ‘Cheyenne’, and ‘Comanche’, which all have the same parentage. The MPS dendrogram did reflect what is known of the Arkansas genotypes, showing close relationships between parents and offspring (such as ‘Navaho’/‘Apache’, ‘Arapaho’/‘Prime-Jim’<sup>TM</sup>, and ‘Choctaw’/‘Rosborough’). The majority of the closest relationships had either ‘Brazos’ and/or ‘Darrow’ in their lineages and was represented in the 18 genotypes at the top of the dendrogram (data not shown). The cophenetic correlation derived from the Mantel (1967) test was  $r = 0.86$ , suggesting a good fit of the dendrogram to the similarity matrix (Rohlf, 2000). The CR dendrogram also yielded a good fit,  $r = 0.82$  and was similar to the MPS dendrogram.

Maximum potential similarity produced similarity coefficients that were very close to those of RFLP and RAPD studies and was much closer to values produced by the molecular studies than were the coefficients of relationship. In several instances the minisatellite (RFLP) data from Nybom et al. (1989) and the RAPD data from Stafne et al. (2003) were within 0.05 of the MPS, though were more disparate from CR values in most instances (Table 4).

Table 4. Direct comparisons of molecular analysis and genetic similarity among blackberry cultivars common to all studies

Cultivar pair	RFLP <sup>z</sup>	RAPD <sup>y</sup>	MPS <sup>x</sup>	CR <sup>w</sup>
Brazos-				
Darrow	0.53	0.55	0.50	0.13
Choctaw	0.84	0.64	0.63	0.53
Rosborough	0.84	0.61	0.75	0.50
Shawnee	0.76	0.62	0.66	0.54
Darrow-				
Choctaw	0.67	0.61	0.50	0.38
Rosborough	0.56	0.53	0.38	0.06
Shawnee	0.80	0.53	0.56	0.41
Choctaw-				
Rosborough	0.89	0.51	0.88	0.64
Shawnee	0.80	0.63	0.81	0.39
Rosborough-				
Shawnee	0.70	0.70	0.69	0.30

<sup>z</sup>Nybom et al. (1989).

<sup>y</sup>Stafne et al. (2003).

<sup>x</sup>Maximum potential similarity.

<sup>w</sup>Coefficient of relationship.

## Discussion

Nineteen founding clones were identified, of which six are either named cultivars or selections that have unknown parentage information. Because of this, it is possible that GC percentages are underestimated due to the likelihood that these genotypes contain some or all of the other founding clone species within them. This would most likely increase the mean GC percentage of a few species, especially those in the top seven positions in Table 2, and generally insinuate even narrower genetic diversity within this sample of 32 cultivars. The highly prevalent founding clones *R. allegheniensis*, *R. argutus*, and *R. frondosus* contributed most of the genetic background to the group of 32 cultivars in this study, nearly 50%.

The genetic diversity within this sample of 32 cultivars can be attributed to species such as, *R. strigosus* Michx. and *R. occidentalis* which are not blackberries, but rather raspberry species from North America. *R. strigosus* is a red raspberry and *R. occidentalis* is a black raspberry. Other diverse species include *R. thyrsiger* Banning and Focke and *R. ulmifolius* var. *inermis* Focke, both European blackberries. *R. thyrsiger* has excellent flavor and good cluster size and *R. ulmifolius* var. *inermis* has been used to transmit the important recessive thornless gene to modern blackberry cultivars through ‘John Innes’, ‘Thornfree’, and ‘Merton Thornless’. ‘Crisfield’, a selection of *R. cuneifolius* Pursh. is the seed parent of the recent cultivar release ‘Chesapeake’. Hedrick (1925) described *R. cuneifolius* as one of the ‘sand blackberries’ that does well in southern climates. Other cultivars such as ‘Early June’ and ‘Williams’ incorporate ‘Himalaya’, a derivative of a European blackberry, *R. procerus* Muell. However, all of the 32 cultivars in this study have *R. allegheniensis* and/or *R. argutus* in their parental backgrounds except for ‘Brewer’, which is a cross between *R. pergratus* Blanch. and *R. frondosus* (Hedrick, 1925).

Some cultivars such as ‘Mersereau’, ‘Mayes’, and ‘Brewer’ do not contain *R. allegheniensis* in their respective backgrounds and ‘Lawton’ contains no *R. argutus*. ‘Eldorado’ contains significant amounts of both *R. allegheniensis* and *R. argutus*, but no other species. Therefore, it has a higher level of *R. allegheniensis* than most other cultivars (except ‘Lawton’) and more *R. argutus* (except ‘Mayes’ and ‘Mersereau’). Founding clones such as ‘Georgia Mammoth’, ‘Dew-black’, and ‘Well’s Beauty’ have unknown parentages, but are likely closely related to known genotypes. ‘Well’s Beauty’ is probably very

similar to 'Brazos' (J.N. Moore, personal communication), but was treated as a founding clone in this study. Cluster analysis (data now shown) did not bear out definitive clusters based on breeding program or founding clone species. This is not an unexpected result, as all the cultivars are from eastern North America and most contain some or all of the same founding clones. Alice and Campbell (1999) reported that internal transcribed spacer (ITS) region sequence divergence between species of the blackberry subgenus averaged only 1.2%. Stafne et al. (2004) found this to be true at the cultivar level as well, with little distinguishing sequence differentiation in the ITS region among 'Apache', 'Arapaho', 'Brazos', 'Illini Hardy', and 'Prime-Jim'<sup>TM</sup>.

The analysis of inbreeding coefficients is based upon accurate pedigree information, but sometimes that information is questionable, or even, unavailable. Therefore, the inbreeding estimates of this study may be too low in some cases because it is likely that some of the founding clones arose from other founding clones and therefore the total number of parents in the breeding population would be inflated leading to reduced inbreeding coefficients (Sjulin & Dale, 1987). Also, open-pollinations were considered to have an unknown male parent. If the open-pollination was actually a self of the seed parent, then the inbreeding would be higher. Therefore, some of the inbreeding coefficients in this study may be lower than reality in those cultivars that have an open-pollinated generation.

Higher inbreeding coefficients neither necessarily suggest a narrow genetic base nor a high degree of homozygosity (Dale et al., 1993), because the genetic base of blackberries is varied, inclusive of several species. Jennings (1967, 1972) also reported that the heterozygote has a selective advantage in red raspberry and this may be case for blackberries as well. Within the University of Arkansas blackberry-breeding program 'Darrow' and 'Brazos' were used extensively because of their proclivity for producing exceptional offspring and because the need to recover recessive traits like thornlessness and primocane fruiting necessitated backcross breeding, thus increasing inbreeding coefficients. The higher inbreeding coefficients seen in the University of Arkansas cultivars may also be due to the duration of the blackberry-breeding program. More generations of hybridization can lead to higher inbreeding coefficients (Sjulin & Dale, 1987) and this would explain the discrepancy between the Arkansas cultivars and the non-Arkansas cultivars.

Ostensibly, founding clones should contribute the sum total of all genes involved within a particular genotype. The MPS coefficient estimate is higher than that of the CR likely due to the fact that CR incorporates intermediate ancestors, including those that are unshared between genotypes thus allowing for the possibility of those genotypes contributing more than is accounted for in the MPS calculation. The Arkansas cultivars have a higher average MPS (0.50) when compared to the non-Arkansas cultivars in this study (0.32). When broken down into geographic regions, the cultivars excluding Arkansas range from an average MPS of 0.29 to 0.33. Most of the non-Arkansas cultivars in this study were released since 1957, but do not have the same level of intensive breeding that has been used in the Arkansas program. The University of Arkansas blackberry-breeding program has existed for 40 years resulting in several cycles of breeding. Arkansas cultivars Apache, Navaho, Prime-Jan<sup>TM</sup>, and Prime-Jim<sup>TM</sup> all have nine generations in their lineage and 'Arapaho', 'Chickasaw', 'Kiowa', and 'Ouachita' have eight generations. Of the other cultivars in this study, only 'Illini Hardy' has even eight generations. Thus, the reason for higher inbreeding coefficients and MPS coefficients is associated with more generations of crossing related material.

Recently, molecular studies to determine genetic relatedness have been reported in *Rubus* (Graham & McNicol, 1995; Graham et al., 1994; Graham et al., 1997; Nybom & Hall, 1991; Pamfil et al., 2000; Trolepe & Moore, 1999), however few have dealt directly with blackberry cultivars (Nybom et al., 1989; Stafne et al., 2003). Even though the advent of molecular techniques has made genetic similarity results more robust, it is still of interest to determine how established pedigrees of blackberry genotypes relate to each other and what the potential ramifications are for future breeding objectives.

Not all blackberry genotypes have been subjected to molecular analysis and some older cultivars have been lost, and therefore are unable to be analyzed by molecular techniques. Since blackberries are not considered one of the major fruit crops worldwide, a lack of studies involving molecular markers have been done, though more are being initiated (Lewers et al., 2004). In Table 4, some comparisons are made between cultivars that have been subjected to molecular marker studies. If molecular marker analysis is to be considered the most accurate depiction of genetic similarity, then CR appears to underestimate relatedness in all cases. This may be due to the inference that all intermediate

ancestors are unrelated. However, MPS does much better than CR in estimating pairwise genetic similarity when compared with molecular studies.

## Conclusions

New germplasm could infuse genes for future breeding endeavors, thus widening the genetic base of cultivated blackberry. An introduction of new germplasm could provide new genes for breeding purposes and the University of Arkansas blackberry-breeding program maintains an extensive variety of genes from which to continue its production of improved blackberry cultivars. Even though there appears to be a significant amount of inbreeding within the Arkansas germplasm, it was a result of recovering recessive alleles and a primary dependence upon Texas A&M-developed germplasm in the early years of the program. These factors have led to high similarity coefficients among the 13 Arkansas blackberry cultivars in this study. It appears that with more generations of hybridization, the higher the level of inbreeding, though there is still a wide diversity of genes to explore within the program.

Ultimately, for maximization of genetic progress in breeding, the initial breeding genetic base should be broad and many, non-related parents should be used within each round of hybridization to diminish inbreeding and a reduction of desirable genes through genetic drift. Another way to decrease inbreeding is to introduce new germplasm into the breeding population. This could include unrelated improved genotypes and/or wild selections. Darrow (1937) mentions several species that have superior qualities that are not included in the list of founding clones for this study. For example, he points out that *R. borrieri* T.B. Salter is early ripening and *R. nitidioides* W.C.R. Watson has large flower clusters. Darrow (1967) also states that *R. canadensis* L. would be a viable species for broadening the germplasm base for breeding hardy, thornless blackberries. Although inbreeding is apparent in some cultivars in this study, there appears to be enough overall genetic diversity to continue breeding without significant, imminent concern for loss of diversity.

Maximum potential similarity (MPS) appears to have some validity for gauging similarity between blackberry cultivars, with the exception of those that have identical parentage. Also, the semblance between the MPS and molecular results may suggest that bivalent chromosome pairing does occur in blackberries, at least in some crosses, as MPS is based on that hy-

pothesis. Therefore, MPS provides a quick and relatively accurate way to assess genetic relatedness among genotypes with known parentages. However, any assessment should be supported by other methods of estimation, such as molecular studies, because of the possibility of incorrect parentage records and the cytological anomalies present in blackberry. An exhaustive study of the hundreds of blackberry cultivars released since the mid-1800s could yield more cluster definition and, in tandem with molecular studies, unknown founding clones could be potentially identified to clarify genotype relatedness issues.

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