Simple Sequence Repeat (SSR) Markers for Genetic Mapping of Raspberry and Blackberry

Eric T. Stafne and John R. Clark
Department of Horticulture, University of Arkansas, Fayetteville, AR 72701

Courtney A. Weber
Department of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456

Julie Graham
Scottish Crop Research Institute, Invergowrie, Dundee, Scotland DD2 5DA, UK

Kim S. Lewers[1]
USDA–ARS Fruit Laboratory, 10300 Baltimore Avenue, BARC-West Building 010A, Beltsville, MD 20705

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Abstract. **Interest in molecular markers and genetic maps is growing among researchers developing new cultivars of *Rubus* L. (raspberry and blackberry). Several traits of interest fail to express in seedlings or reliably in some environments and are candidates for marker-assisted selection. A growing number of simple sequence repeat (SSR) molecular markers derived from *Rubus* and *Fragaria* L. (strawberry) are available for use with *Rubus* mapping populations. The objectives of this study were to test 142 of these SSR markers to screen raspberry and blackberry parental genotypes for potential use in existing mapping populations that segregate for traits of interest, determine the extent of inter-species and inter-genera transferability with amplification, and determine the level of polymorphism among the parents. Up to 32 of the SSR primer pairs tested may be useful for genetic mapping in both the blackberry population and at least one of the raspberry populations. The maximum number of SSR primer pairs found useable for mapping was 60 for the raspberry population and 45 for the blackberry population. Acquisition of many more nucleotide sequences from red raspberry, black raspberry, and blackberry are required to develop useful molecular markers and genetic maps for these species. *Rubus*, family Rosaceae, is a highly diverse genus that contains hundreds of heterozygous species. The family is one of the most agronomically important plant families in temperate regions of the world (Dirlewanger et al., 2002), although they also occur in tropical and arctic regions as well. The most important commercial subgenus of *Rubus* is *Idaeobatus* Focke, the raspberries, which are primarily diploids. This subgenus contains the european red raspberry *R. idaeus* ssp. *idaeus* L., as well as the american black raspberry *R. occidentalis* L. and the american red raspberry *R. idaeus* ssp. *strigosus* Michx. Interspecific hybridization of these, and other raspberry species, has led to greater genetic diversity and allowed for the introgression of superior traits such as large fruit size, fruit firmness and quality, disease resistance, and winter hardiness.**

Blackberries reside in the subgenus *Rubus* (formerly *Eubatus* Focke). Modern eastern north american blackberry cultivars are comprised of many species, including *R. allegheniensis* Porter, *R. argutus* Link., *R. cuneifolius* Pursh., *R. frondosus* Bigel., *R. rubrisetus* Rydb. (=*R. trivialis* L.), and *R. pergratus* Blanch., as well as european blackberry species such as *R. ulmifolius* var. *inermis* Focke, *R. thyrsiger* Banning and Focke, and *R. procerus* P.J. Muell. Blackberry is a highly heterozygous plant and many current cultivars are tetraploid. Interest in development of molecular markers and maps has been limited, 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. Also, polyploid plants often display problems with reproducibility and amplification in polymerase chain reactions (PCR) (Buteler et al., 1999; Diwan et al., 1997; Röder et al., 1995). However, because eastern north american blackberries for the most part are not apomictic (Hall, 1990), sexual recombination can be assumed. Although alloploidy in *Rubus* is more common than autoploidy (Ourecky, 1975), various degrees of meiotic irregularity occur in north american species, and the high percentage of multivalents observed in tetraploid species suggests that both auto- and allo-polyploidy are involved (Einset, 1947). The desire to incorporate relatively new and exciting traits of commercial importance such as thornlessness and fruit production on first-year canes (primocane fruiting) has led to greater interest in molecular marker analysis in blackberries.

Simple sequence repeat (SSR) markers are repeats of short nucleotide sequences, usually equal to or less than six bases in length, that vary in number (Rafalski et al., 1996). SSR markers have become quite useful in various aspects of molecular genetic studies in the past decade, including assessment of

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[1]To whom reprint requests should be addressed. E-mail: lewersk@ba.ars.usda.gov.

genetic diversity (Amsellem et al., 2001; Ashley et al., 2003), fingerprinting (Rongwen et al., 1995), ecological-genetic studies (Li et al., 2000), marker-assisted selection (Fazio et al., 2003), gene flow characterization (Aldrich and Hamrick, 1998; Chase et al., 1996), and genetic linkage mapping (Akkaya et al., 1995; Broun and Tanksley, 1996). They are desirable because they are often codominant, highly reproducible, frequent in most eukaryotes, and reveal high allelic diversity (Mohan et al., 1997). SSR markers are amplified using the PCR, thus allowing for the rapid generation of data from a relatively small amount of plant tissue. However, SSR markers are also expensive to generate and can be very laborious in certain species. Because of these expense and labor issues, many researchers have attempted to use SSR primers developed from one species for studies on related species and genera. Several studies have indicated successful inter-species and inter-genera results within Rosaceae (Ashley et al., 2003; Cipriani et al., 1999; Decroocq et al., 2003; Dirlewanger et al., 2002; Graham et al., 2002; Lewers et al., 2005) and other plant families (Bowers et al., 1996; Cordeiro et al., 2001; Decroocq et al., 2003; Echt et al., 1999; Guilford et al., 1997; Huang et al., 1998; Kijas et al., 1997; Selvi et al., 2003; Sharon et al., 1997).

One objective of this study was to utilize SSR markers developed from red raspberry [*R. idaeus* ssp. *idaeus* 'Glen Moy' (Graham et al., 2002, 2004)], wild bramble [*R. alceifolius* Poir (Amsellem et al., 2001)], and strawberry [*F.* ×*ananassa* Duschene ex Rozier 'Earliglow' (Lewers et al., 2005); *F. virginiana* Mill. (Ashley et al., 2003); and *Fragaria vesca* L. (James et al., 2003)] to screen raspberry and blackberry parental genotypes for potential use in mapping populations that segregate for important traits of interest and determine the level of polymorphism among the parents. A second objective was to determine the extent of inter-species and inter-genera transferability with amplification. Success in this area would circumvent the need for further creation of SSR markers specific to black raspberry and blackberry, thus greatly reducing SSR marker-development expenditures and allowing genetic mapping using non-species-specific SSR markers.

Materials and Methods

Description of mapping populations. A black raspberry × red raspberry cross of 'Jewel' × NY322 was made to create an interspecific hybrid that allows for the development of a map of each parent, and thus each species, in the F_1 generation. There is no recombination in the F_1 between the two genomes and the heterozygosity of each parent can be mapped. The F_1 population segregates for primocane fruiting, root production of adventitious shoots (suckering), tip rooting, and other important traits that would be good candidates for marker-assisted selection in a raspberry breeding program. An F_2 population can then be used to join the maps based on recombination in that generation. SSR markers have proven successful in similar studies in the past (Lorieux et al., 2000). The breeding selection NY322 also carries a recessive allele for yellow fruit color that can be mapped in the F_2 population.

The blackberry × blackberry cross of 'APF-12' (Prime-Jim) × 'Arapaho' was made to produce F_1 progeny that segregate for primocane fruiting, thornlessness, and other traits. Release of the first commercial primocane-fruiting blackberry cultivar (Clark et al., 2004), increased the importance of understanding more about the inheritance and location of the trait within the blackberry genome. Primocane fruiting does not always express in the first year and, therefore, is a good candidate for marker-assisted selection. Previous work has indicated that the trait is qualitative, recessive, and segregates as would be expected in an autotetraploid (Lopez-Medina et al., 2000).

Description of SSR primer pairs. SSR primers tested for utility in the raspberry and blackberry mapping populations included 84 primer pairs derived from the spineless red raspberry 'Glen Moy' released by the Scottish Crops Research Institute in 1981 (Graham et al., 2002, 2004). As with many raspberry cultivars, the pedigree of 'Glen Moy' is complex and includes *R. idaeus* ssp. *idaeus*, *R. occidentalis*, and *R. ideaus* ssp. *strigosus* (Daubney, 1997), and SSR primer pairs derived from 'Glen Moy' sequence should reflect to some extent the species in its pedigree. Eight other *Rubus*-derived SSR primer pairs came from *R. alceifolius* Poir., a European weed (Amsellem et al., 2001). Because *Rubus* is classified in the same subfamily (Rosoideae) as *Fragaria* (strawberry), 57 strawberry-derived SSR primer pairs also were tested. A total of 39 SSR primer pairs came from genomic and expressed sequence tags (ESTs) from *F.* ×*ananassa*, cultivated strawberry (Lewers et al., 2005); four SSR primer pairs came from *F. virginiana*, the paternal progenitor of the cultivated strawberry (Ashley et al., 2003), and 14 SSR primer pairs came from the diploid *F. vesca* (James et al., 2003; Lewers et al., 2005).

PCR and electrophoresis. To determine how useful the primers would be for genetic mapping in *Rubus*, parents of the raspberry mapping population ('Jewel' and NY322) and the blackberry mapping population, ('Arapaho' and 'APF-12') were used in PCR to test the primer pairs' abilities to amplify a product and to detect polymorphisms. Young leaves were harvested from greenhouse- or field-grown plants, and DNA was extracted using a DNeasy Plant Maxi Kit (Qiagen, Valencia, Calif.). DNA was quantified using the Spectramax 190 spectrophotometer (Molecular Devices, Sunnyvale, Calif.), and diluted with 1× TE buffer to 30 $ng \cdot \mu L^{-1}$. Dilutions to 0.03 $ng \cdot \mu L^{-1}$ were made with ddH_2O.

To test if the primer pairs would amplify a product, unlabeled primers (Qiagen) were used in PCR. The reaction components included 1× PCR buffer with 1.5 mM $MgCl_2$ (Qiagen), 0.67 mM dNTPs, 0.5 μM each primer, 0.4 U HotStar Taq polymerase enzyme (Qiagen), and template DNA at 0.002 $ng \cdot \mu L^{-1}$ in a total reaction volume of 15 μL. An MJ Research Engine Tetrad with 96-well blocks (MJ Research, Waltham, Mass.) was used for amplification, heating reaction components to 95 °C for 15 min to activate the polymerase, followed by 30 cycles of 40 s at 94 °C, 40 s at 52 °C or 59 °C, and 40 s at 72 °C. A final 10-min extension period at 72 °C was followed by storage at 4 °C. PCR products (5 μL) were added to water (15 μL) and visualized after 12 min of electrophoresis through a 2% agarose gel using the E-gel 96 system (Invitrogen Corp., Carlsbad, Calif.). Gel images were captured in TIF files using an Alpha-Imager (Alpha Innotech Corp., San Leandro, Calif.), and analyzed with the E-gel 96 Editor software (Invitrogen).

To size the PCR products and identify size-polymorphisms, "poor man's" PCR reactions were used as described by Schuelke (2000). Three primers of unequal proportion are used in each reaction. The first 30 cycles use an annealing temperature specific to the genomic target sequence while the final eight cycles use a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned to incorporate a fluorescent protein into the amplification products. The M13 sequence, 5´TGTAAAACGACGGCCAGT3´ was added to the 5´ ends of the forward primers. For cost efficiency, M13 primers were labeled with the fluorescent proteins, FAM, VIC, NED, and PET (Applied Biosystems, Foster City, Calif.). Reac-

tion components were assembled the same as for the unlabeled reactions, though three primers are used in each reaction: The redesigned forward primer, the reverse primer, and the fluorescently labeled M13 primer. The primer concentrations were in the proportions described by Schuelke (2000); the unlabeled reverse primer concentrations and the labeled M13 primer concentrations were equimolar at 0.5 μM, while the un-labeled chimeric forward primer concentration was one fourth the molarity of the other two primers, 0.125 μM. The reaction components were heated the same as for the un-labeled reactions, but followed by eight cycles of 40 s at 94 °C, 40 s at 52 °C, and 40 s at 72 °C to incorporate the fluorescently labeled M13 primer, and the final 10 min extension period at 72 °C and storage at 4 °C. Fluorescently labeled PCR products were separated by capillary gel electrophoresis and detected using the Applied Biosystems, 3730 DNA Analyzer. The size standard used during electrophoresis was GENESCAN 500HD (LIZ). Sizing data were analyzed using the Applied Biosystems Genemapper software.

Results

A total of 142 unique SSR primer pairs were used in PCR amplifications with the four *Rubus* genotypes (Table 1); seven pairs of the raspberry-derived SSR primer pairs were identical. The percentages of 'Glen Moy' red raspberry-derived SSR primer pairs that amplified a product were 65% for red raspberry selection NY322, 47% for black raspberry 'Jewel', 29% for blackberry 'APF-12', and 30% for blackberry 'Arapaho'. Likewise, 32% and 27% of the strawberry-derived SSR primer pairs amplified a product from blackberry 'APF-12' and 'Arapaho', respectively. Of the eight SSR primer pairs derived from *R. alceifolius*, two (25%) amplified products from all four parents. This was slightly less than the percentage of raspberry- or strawberry-derived primer pairs that amplified products from the blackberry parents. Only 12% of the strawberry-derived SSR primer pairs amplified a product from either NY322 or 'Jewel' raspberry.

Our findings indicate that 51 raspberry-derived SSR primer pairs, two *R. alceifolius* SSR primer pairs, and seven strawberry-derived SSR primer pairs, for a total of 60 SSR primer pairs are available for genetic mapping in the raspberry population. Twenty-six of the raspberry-derived SSR primer pairs, both of the *R. alceifolius* SSR primer pairs, and one of the strawberry-derived SSR primer pairs, for a total of 29 SSR primer pairs detected product size differences between NY322 and 'Jewel'. Sixteen of the raspberry-derived SSR primer pairs amplified a product from one parent but not the other. Nine raspberry-derived SSR primer pairs and six more strawberry-derived SSR primer pairs amplified monomorphic products from the raspberry parents. These 15 SSR primer pairs may be useful for mapping in the F_2 population since the F_1 individual used to generate the F_2 population may be heterozygous at several loci.

Twenty-four raspberry-derived SSR primer pairs, both the *R. alceifolius* SSR, and 19 strawberry-derived SSR primer pairs, for a total of 45 SSR primer pairs are available for genetic mapping in the blackberry population. Fourteen of the raspberry-derived SSR primer pairs, one of the *R. alceifolius* SSR primer pairs, and four of the strawberry-derived SSR primer pairs, for a total of 19 SSR primer pair, detected base-pair differences between 'APF-12' and 'Arapaho'. Three of the raspberry-derived SSR primer pairs and three of the strawberry-derived SSR primer pairs amplified a product from one but not the other parent. Seven raspberry-derived SSR primer pairs, one *R. alceifolius* SSR, and 12 strawberry-derived SSR primer pairs amplified monomorphic products from the blackberry parents. These 20 SSR primer pairs may be useful for mapping since blackberry is highly heterozygous and is autotetrasomic (Lopez-Medina et al., 2000).

Notably, a total of 32 SSR primer pairs are available for genetic mapping in these raspberry and blackberry populations, 23 derived from raspberry, 2 from *R. alceifolius* SSR, and 7 from strawberry. Of these, 22 detected product size polymorphisms between at least one set of population parents, and 10, mostly raspberry-derived, detected polymorphisms between both sets. Twenty SSRs amplified monomorphic products between at least one set of parents, and seven of these, mainly from strawberry, amplified monomorphic products in both sets.

Discussion

A useful number of SSR primer pairs derived from red raspberry 'Glen Moy' amplified a product from the parents of the raspberry populations, but the percentage was much lower than what has been reported among strawberry species (Ashley et al., 2003; Lewers et al., 2005). The genetic background of 'Glen Moy' is quite diverse, but >80% *R. idaeus* ssp. *idaeus* with the remainder made up of 12% *R. idaeus* ssp. *strigosus* and 2% *R. occidentalis* (Dale et al., 1993). Of the raspberry genotypes tested, NY322 is comprised of more than 52% *R. idaeus* ssp. *idaeus*, however, it also has significantly more *R. idaeus* ssp. *strigosus* than does 'Glen Moy'. Nearly 29% of NY322 is from *R. idaeus* ssp. *strigosus*-based genetic material. In addition to those two species, *R. pungens oldhamii* Miq. and *R. chamaemorus* L. are also present in its background (Dale et al., 1993). The large disparity in the amount of non-*R. idaeus* ssp. *idaeus* genes between 'Glen Moy' and NY322 could account for the lower percentage of useful SSR primer pairs. Also, only three founding clones are shared between the two genotypes, including 'Lloyd George', 'Newman', and 'Herbert'. NY322 and 'Glen Moy' have roughly equal amounts of 'Lloyd George' and 'Herbert' in their respective backgrounds, but NY322 has much more genetic material donated from 'Newman'.

Howarth et al. (1997) found 99% sequence identity between *R. idaeus* ssp. *idaeus* and *R. idaeus* ssp. *strigosus* for the *ndh*F gene and stated that *R. idaeus* ssp. *idaeus* formed a cohesive group with *R. idaeus* ssp. *stigosus* and another North American subspecies [*R. melanolasius* (Dieck) Focke]. That study was based on a very specific region of chloroplast DNA and may not be variable enough to gauge the diversity of *Rubus* species and subspecies. However, a separate study conducted by Moore (1993) showed that cultivars could be placed into distinct groupings based on species background for chloroplast DNA. The *R. idaeus* ssp. *strigosus* cultivar Dalhousie Lake 4 comprised a group unto itself based on banding patterns. It was more closely related to the *R. idaeus* groups than to the *R. occidentalis* groups. Waugh et al. (1990) found no differences in chloroplast DNA among *R. idaeus* ssp. *idaeus* genotypes and Moore (1993) found few differences, suggesting that there is little chloroplast DNA variability within *R. idaeus* ssp. *idaeus*, but both stated that nuclear DNA might be more sensitive to variation.

A study by Graham and McNicol (1995) using RAPD markers found that *R. idaeus* ssp. *idaeus* clones clustered differently from those of *R. occidentalis*. One *R. idaeus* ssp. *strigosus* clone ('Latham') was included and clustered with the *R. idaeus* ssp. *idaeus* genotypes, but was the most distinct of the red raspberry group. Overall, *R. occidentalis* had a low similarity index when

Table 1. Previously published simple sequence repeat molecular markers (SSRs) tested in polymerase chain reactions (PCR) with template DNA extracted from parents of *Rubus* (raspberry and blackberry) genetic mapping populations to determine potential utility for genetic mapping within the populations. A total of 142 SSR primer pairs from the cited manuscripts were tested, and those not in this table did not amplify a product in a minimum of four attempts. Breeding selection NY322 (*R. idaeus,* red raspberry) and 'Jewel' (*R. occidentalis*, black raspberry) were used to derive an F_1 and an F_2 population. 'Arapaho' (*Rubus* spp., blackberry) and 'APF-12' (*R.* spp., blackberry) also were used to derive an F_1 population.

SSR primer pair[z]	NY322 PCR product size	'Jewel' PCR product size	Type or presence of polymorphism	'Arapaho' PCR product size	'APF-12' PCR product size	Type or presence of polymorphism
Derived from *Rubus* species hybrid red raspberry 'Glen Moy' (Graham et al., 2002, 2004)						
Rubus 262 and Rubus 262b	242	220	product size	219/225/266	219/225/261	product size
Rubus 277 and Rubus 277a	250	250	monomorphic	215/269/281	207/269/281	product size
Rubus 26 and Rubus 26a	107/138	138	product size	none	none	none
Rubus126 and Rubus 126b	215	155/165	product size	none	none	none
Rubus 223 and Rubus 223a	171	121/155	product size	none	none	none
Rubus 280 and Rubus 260a	165	none	amplification	none	none	none
Rubus 2a	174/182/193	182	product size	179/214	179/214	monomorphic
Rubus 6a	150	150	monomorphic	none	none	none
Rubus12a	160	141	product size	247/291/302/339	229/247/302/345	product size
Rubus16a	169	169	monomorphic	164	none	amplification
Rubus 22a	134/169	126/134/169	product size	none	none	none
Rubus 24a	184	none	amplification	133	133/168	product size
Rubus 25a	163/173	173	product size	none	none	none
Rubus 35a	229/239	256	product size	none	none	none
Rubus 43a	226	226	monomorphic	219/227	212/219/227	product size
Rubus 45c	none	none	none	184	none	amplification
Rubus 47a	232	none	amplification	217/238/248	217/238/248	monomorphic
Rubus 57a	191	none	amplification	none	none	none
Rubus 59b	220/259	none	amplification	none	none	none
Rubus 98d	198	198	monomorphic	none	none	none
Rubus 102c	137	137	monomorphic	none	137	amplification
Rubus 105b	165/173/181	165	product size	171/173	175	product size
Rubus 107a	172/179	179	product size	190/195	190	product size
Rubus 110a	176/194	186/201	product size	203	203	monomorphic
Rubus 116a	299	none	amplification	none	none	none
Rubus 117b	132/134/158	132/147/153/273	product size	127/147/162	127/151/162	product size
Rubus 118b	129/180	none	amplification	none	none	none
Rubus 119a	157	157/176	product size	157	157	monomorphic
Rubus 123a	158/168	158/172	product size	none	none	none
Rubus 137a	198	none	amplification	none	none	none
Rubus 145a	152	152	monomorphic	150/154	145/150	product size
Rubus 160a	173	179	product size	none	none	none
Rubus 167a	198	178	product size	none	none	none
Rubus 194h	200/202	143/145	product size	127/154	127	product size
Rubus 210a	122/130	none	amplification	none	none	none
Rubus 228a	159/161/163/165	142/149	product size	none	none	none
Rubus 237b	160	157	product size	153	157	product size
Rubus 251a and Rubus 270a	182/206	182	product size	197/206	206	product size
Rubus 253a	170/191	none	amplification	none	none	none
Rubus 256e	209	167	product size	none	none	none
Rubus 257a	203	207	product size	none	none	none
Rubus 259f	265	257	product size	261	261	monomorphic
Rubus 263f	none	254	amplification	none	none	none
Rubus 264b	193	none	amplification	none	none	none
Rubus 268b	204/226	204	product size	none	none	none
Rubus 275a	161	161	monomorphic	182/206	140/172/182	product size
Rubus 285a	188/205	none	amplification	196/217/240	196/232	product size
Rubus 289a	150	none	amplification	none	none	none
RubfruitE4	182	182	monomorphic	182	182	monomorphic
RubfruitC1	172	177/180	product size	172	172	monomorphic
Rubleaf86	253/255	none	amplification	none	none	none
Rubleaf97	222	none	amplification	none	none	none

continued next page

Table 1. Continued.

SSR primer pair[z]	NY322 PCR product size	'Jewel' PCR product size	Type or presence of polymorphism	'Arapaho' PCR product size	'APF-12' PCR product size	Type or presence of polymorphism
Derived from *Rubus alceifolius* bramble (Amsellem et al., 2001)						
mRaCIRRI1D3	217	195/212	product size	213	213	monomorphic
mRaCIRRIV2A8	209/229	216/223	product size	218/223/228	198/218/223	product size
Derived from *Fragaria* ×*ananassa* strawberry (Lewers et al., 2005)						
ARSFL_4	none	none	none	171	171	monomorphic
ARSFL_7	202	202	monomorphic	202	202	monomorphic
ARSFL_9	118/198/210/238	188/198/210	product size	219/228/234/242	208/222/225/239	product size
ARSFL_10	none	none	none	267	161/242	product size
ARSFL_11	none	none	none	255	255	monomorphic
ARSFL_13	none	none	none	none	185	amplification
ARSFL_22	none	none	none	183/187	183/187	monomorphic
ARSFL_23	none	none	none	165/171	168	product size
ARSFL_30	174	174	monomorphic	174/178	174/178	monomorphic
ARSFL_34	none	none	none	327	327	monomorphic
ARSFL_97	none	none	none	184	184	monomorphic
ARSFL_104	198	198	monomorphic	200	200	monomorphic
ARSFL_133	204	204	monomorphic	189/204	189/204	monomorphic
ARSFL_134	182/232	182/232	monomorphic	182/232	182/232	monomorphic
Derived from *Fragaria virginiana* strawberry (Ashley et al., 2003)						
Fvi11	none	none	none	none	142	amplification
Fvi20	132	132	monomorphic	132	132	monomorphic
Derived from *Fragaria vesca* strawberry (James et al., 2003)						
EMFv4	none	none	none	242/260/302/365	220/260/365	monomorphic
EMFv8	none	none	none	265/317	165/213	product size
EMFv9	none	none	none	none	218/224	amplification

[z]If two SSR primer pairs are listed together on a line, we found their sequences were identical.

compared to both *R. idaeus* ssp. *idaeus* 'Glen Moy' and *R. idaeus* ssp. *strigosus* 'Latham' (39% and 42%, respectively). 'Latham' was more similar to 'Glen Moy' (53%) than to *R. occidentalis* in that study, however, a previous experiment (Graham et al., 1994) showed less similarity (40%) between the two cultivars. Trople and Moore (1999) found that *R. occidentalis* was not closely related to either *R. idaeus* ssp. *strigosus* or *R. idaeus* ssp. *idaeus* based on similarity values derived from RAPDs (31% to 35%). Yet two *R. idaeus* ssp. *idaeus* cultivars, Meeker and Tulameen, had a high genetic similarity of 77%. The *R. idaeus* ssp. *strigosus* cultivar Dalhousie Lake 4 was only slightly more similar to the *R. idaeus* ssp. *idaeus* cultivars (40% to 45%).

As for 'Jewel', a black raspberry totally derived from *R. occidentalis* (Ourecky and Slate, 1973), it seems that 47% amplification using 'Glen Moy' SSR primer pairs is better than could be expected, especially since 'Glen Moy' only has 2% *R. occidentalis* in its background (through 'Cumberland'). This result reveals that the use of cross-species SSR primer pairs is possible within *Rubus*, although not as successful as in other genera such as *Fragaria* (Ashley et al., 2003; Lewers et al., 2005).

Only one of the eight SSR primer pairs from *R. alceifolius* detected a size polymorphism for NY322 and 'Jewel.' Another SSR was monomorphic, thus only 25% of the SSR primer pairs from the wild bramble species *R. alceifolius* amplified any product. *Rubus alceifolius* is in the *Rubus* subgenus, *Malachobatus* Focke, which primarily occurs in Asia. The probable divergent evolution of this species contributed to the failure of most of the SSR markers. *Rubus alceifolius* is native to Southeast Asia and has been introduced to several areas where it has become a weed species. However, the derivation of the original SSR primer sequences was from plants from La Réunion, an island east of Madagascar in the Indian Ocean. And although amplification was found with other species from the *Rubus* subgenera *Idaeobatus* and *Malachobatus*, none were tested on subgenus *Rubus*. Results from other studies (Pamfil et al., 2000; Trople and Moore, 1999) suggest that the *Rubus* subgenus is somewhat divergent from both *Idaeobatus* and *Malachobatus*. Cluster analysis has shown that genotypes in the subgenus *Rubus* form distinct clusters from *Malachobatus* and *Idaeobatus*, both of which cluster together (Pamfil et al., 2000). Genetic similarity studies found low percent similarity between species within subgenus *Rubus* and subgenus *Idaeobatus* (Trople and Moore, 1999).

Fewer than expected SSR primer pairs derived from the red raspberry 'Glen Moy' amplified a product from and revealed polymorphism between the blackberry genotypes. This may be attributable to the few shared species within their respective backgrounds. The only known species that is shared between 'Glen Moy' and 'Arapaho' and 'APF-12' is *R. idaeus* ssp. *strigosus*. 'Glen Moy' contains both 'Marlboro' and 'Herbert', both of which are comprised of *R. idaeus* ssp. *strigosus*. For both 'Arapaho' (Moore and Clark, 1993) and 'APF-12', *R. idaeus* ssp. *strigosus* was introduced through the cultivar Brazos *via* Nessberry, which resulted from a cross of *R. trivialis* and 'Brilliant' (*R. idaeus* ssp. *strigosus*). Graham et al. (1994) reported in a study using RAPD markers that the cultivar Latham (derived from *R. idaeus* ssp. *strigosus*) displayed only 30% to 40% similarity to *R. idaeus* ssp. *idaeus*-derived raspberry cultivars. Another study by Trople and Moore (1999) revealed through a similarity index from RAPD markers that the *R. idaeus* ssp. *idaeus*-derived cultivars Meeker and Tulameen were 77% similar, whereas when those two cultivars were compared to a *R. idaeus* ssp. *strigosus* genotype that similarity percentage averaged only 43%.

'Glen Moy' is predominantly made up of *R. idaeus* ssp. *idaeus*, whereas the blackberry cultivars in this study are largely comprised of *R. allegheniensis* and *R. argutus*. In a study that compared sequence data from the internal transcribed spacer region (ITS) of nuclear ribosomal DNA, 'Arapaho' and 'APF-12' showed a genetic distance of 0-1% among other eastern north american blackberry cultivars and species (*R. allegheniensis* and *R. argutus*), but had 3% to 4% distance when compared to *R. idaeus* ssp. *idaeus* and 'Heritage', a red raspberry cultivar (Stafne et al., 2004). Other studies found similar results (Alice and Campbell, 1999; Howarth et al., 1997).

Of the eight primer pairs derived from *R. alceifolius*, only two were successful in amplifying a product for the blackberries, 'Arapaho' and 'APF-12'. *Rubus alceifolius* resides in the *Rubus* subgenus, *Malachobatus*, which Pamfil et al. (2000), using RAPD analysis, found to be in mostly distinct clusters, with only *R. parvifolius* L. (*Idaeobatus*) residing in the *Malachobatus* cluster. Three *Malachobatus* species were found in the *Idaeobatus* cluster (*R. hayata-koidzumii* Naruh., *R. assamensis* Focke, and *R. tephrodes* Hance). A study using AFLP markers by Amsellem et al. (2000) reported that a strong separation between *Rubus* subgenus *Idaeobatus* and subgenus *Malachobatus* was evident. They also surmised that the *R. alceifolius* plants, from which the SSR markers were derived, growing on La Réunion, reproduced entirely apomictically, thus resulting in monoclonality. This narrow range of genetic material from which the SSR markers were derived may have contributed to the poor results.

The low percentage success of strawberry-derived SSR markers for the raspberries and blackberries in this study underscores the possibility that cross-generic use of SSR markers may be limited (Lewers et al., 2005). Interestingly, successful intergeneric crosses between a tetraploid blackberry and an octoploid strawberry have been made (Naess et al., 1998), and resulted in a hexaploid plant. This result suggests that some genetic compatibility may exist for fertility, yet the plant had no hybrid characteristics and no difference in chromosome size (Naess et al., 1998). In addition, Lewers et al. (2005) reported that 30 *Rosa*-derived SSR primer pairs failed to amplify a single product from raspberry, blackberry or strawberry genotypes tested, including the four in this study, even though all belong in the subfamily Rosoideae. This is somewhat consistent with prior findings (Decroocq et al., 2003) that indicate limited inter-species transfer of SSR markers within the family Rosaceae. Decroocq et al. (2003) also reported that the threshold distance for amplification within Rosaceae is limited and that the optimal SSR markers were derived from closely related species within the same subgenus for apricot (*Prunus armeniaca* L.).

With a maximum number of SSR primer pairs potentially usable for mapping at only 60 for the raspberry population and only 45 for the blackberry population, there is a clear need for developing additional SSR primer pairs for genetic mapping in both populations. An effort currently is being made to develop more SSR primer pairs from 'Glen Moy' ESTs and genomic sequences. Multiple efforts also are underway to obtain blackberry genomic (N.V. Bassil, personal communication) and EST sequences for SSR development.

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