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

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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 agriculturally 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. rubrissetus* 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-polyplody), and inheritance strategies (disomic and tetrasomic) (Lopez-Medina et al., 2000)] of polyplloid blackberries. Also, polyplloid 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 alloplody in *Rubus* is more common than autopolypeody (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-polyplody are involved (Eisenet, 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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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. vesca ssp. 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 F1 generation. There is no recombination in the F1, between the two genomes and the heterozygosity of each parent can be mapped. The F1 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 F2 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 F2 population.

The blackberry × blackberry cross of ‘APF-12’ (Prime-Jim) × ‘Arapaho’ was made to produce F1 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. ideaeus 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 µL⁻¹. Dilutions to 0.03 ng µL⁻¹ were made with ddH₂O.

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 MgCl₂, 0.67 mM dNTPs, 0.5 µM each primer, 0.4 U HotStar Taq polymerase enzyme (Qiagen), and template DNA at 0.002 ng µL⁻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 with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature. The SSR primer pairs that amplified a product from any of the four parental genotypes were redesigned with a lower annealing temperature.
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 500HE (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. Overall, 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 F₁ population since the F₁ individual used to generate the F₂ population may be heterozygous at several loci.

Twenty-four raspberry-derived SSR primer pairs, both of 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 pairs, 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 ndHF gene and stated that *R. idaeus* ssp. *idaeus* formed a cohesive group with *R. idaeus* ssp. *stigosus* and another North American subspecies [*R. melanolasius* (Dbeck) 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 Dalhousey 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 it 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.

<table>
<thead>
<tr>
<th>SSR primer pair</th>
<th>NY322 PCR product size</th>
<th>‘Jewel’ PCR product size</th>
<th>Type or presence of polymorphism</th>
<th>‘Arapaho’ PCR product size</th>
<th>‘APF-12’ PCR product size</th>
<th>Type or presence of polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Derived from <em>Rubus</em> species hybrid red raspberry ‘Glen Moy’ (Graham et al., 2002, 2004)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rubus 262 and Rubus 262b</td>
<td>242</td>
<td>220</td>
<td>product size</td>
<td>219/225/266</td>
<td>219/225/261</td>
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</tr>
<tr>
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<td>250</td>
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<td>product size</td>
<td>215/269/281</td>
<td>207/269/281</td>
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</tr>
<tr>
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<td>138</td>
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</tr>
<tr>
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<tr>
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<tr>
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<td>none</td>
<td>amplification</td>
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<tr>
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<td>174/182/193</td>
<td>182</td>
<td>product size</td>
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<tr>
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<td>169</td>
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<tr>
<td>Rubus 24a</td>
<td>184</td>
<td>184</td>
<td>product size</td>
<td>133</td>
<td>133/168</td>
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</tr>
<tr>
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<td>186/201</td>
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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 (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.

**Literature Cited**


