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ORIGINAL PAPER

# Construction of black (*Rubus occidentalis*) and red (*R. idaeus*) raspberry linkage maps and their comparison to the genomes of strawberry, apple, and peach

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**Abstract** The genus *Rubus* belongs to the Rosaceae and is comprised of 600–800 species distributed world-wide. To date, genetic maps of the genus consist largely of non-transferable markers such as amplified fragment length polymorphisms. An $F_1$ population developed from a cross between an advanced breeding selection of *Rubus occidentalis* (96395S1) and *R. idaeus* 'Latham' was used to construct a new genetic map consisting of DNA sequence-based markers. The genetic linkage maps presented here are constructed of 131 markers on at least one of the two parental maps. The majority of the markers are orthologous, including 14 Rosaceae conserved orthologous set markers, and 60 new gene-based markers developed for raspberry. Thirty-four published raspberry simple sequence repeat markers were used to align the new maps to published raspberry maps. The 96395S1 genetic map consists of six linkage groups (LG) and covers 309 cM with an average of 10 cM between markers; the 'Latham' genetic map consists of seven LG and covers 561 cM with an average of 5 cM between markers. We used BLAST analysis to align the orthologous sequences used to design primer pairs for *Rubus* genetic mapping with the genome sequences of *Fragaria vesca* 'Hawaii 4', *Malus* × *domestica* 'Golden Delicious', and *Prunus* 'Lovell'. The alignment of the orthologous markers designed here suggests that the genomes of *Rubus* and *Fragaria* have a high degree of synteny and that synteny decreases with phylogenetic distance. Our results give unprecedented insights into the genome evolution of raspberry from the putative ancestral genome of the single ancestor common to Rosaceae.

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## Introduction

The Rosaceae is a large and diverse plant family with many members of economic and nutritional importance. Members of the sub-family Rosoideae, including *Fragaria* (strawberry) and *Rubus* (raspberry and blackberry), have the same base chromosome number of $x = 7$, similar fruit form, and are closely related based on chloroplast and nuclear DNA phylogenies (Potter et al. 2007), as well as morphological data (Eriksson et al. 2003). These lines of evidence suggest colinearity between the *Fragaria* and *Rubus* genomes; however, a lack of transferable markers has made genomic comparisons between these genera unachievable to date (Lewers et al. 2005).

The genus *Rubus*, with an estimated 600–800 species, includes red raspberry (*R. idaeus* L.), black raspberry (*R. occidentalis* L.) both of subgenus *Idaeobatus* (Focke) Focke, and blackberries such as *R. ursinus* Cham. & Schltdl., and *R. laciniatus* Willd., both of subgenus *Rubus* (Thompson 1995b). Sub-family Rosoideae is sister to Spiraeoideae, to which *Malus* (apple) and *Prunus* (peach and other stonefruits) belong (Potter et al. 2007) (Fig. 1). Comparative genetic mapping has been carried out between *Malus* and *Prunus* using restriction fragment length polymorphism (RFLP) markers and isozymes (Dirlewanger et al. 2004); and between *Prunus* and *Fragaria* using RFLP and single nucleotide polymorphism (SNP) markers (Vilanova et al. 2008). A recent comparison of the *Malus* genome to the reference genetic maps of *Prunus* ['Texas' × 'Earlygold', T×E (Joobeur et al. 1998)] and

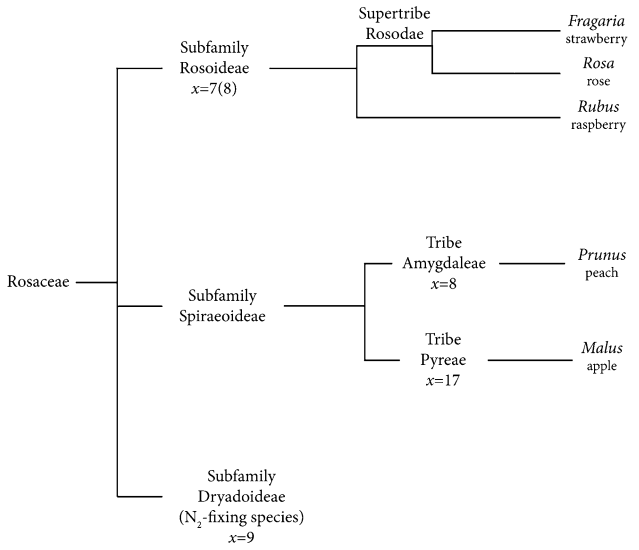



**Fig. 1** Simplified and abbreviated Rosaceae phylogeny. The *diagram* represents the genera included in this study. *Branches* approximate shared ancestry and are not to scale nor all-inclusive. Base chromosome numbers ($x$) are indicated. The phylogeny illustrates the relative distances between the sub-families Rosoideae and Spiraeoideae, and between the genera within each sub-family

*Fragaria* [*F. vesca* × *F. bucharica*, FV×FB (Sargent et al. 2006)] by Illa et al. (2011), and the comparison of genetic linkage maps between *Malus* ['Malling 9' × 'Robusta 5', M.9×R5 (Celton et al. 2009)] and FV×FB (Sargent et al. 2006) by Bushakra et al. (2012) utilized markers designed from transferable Rosaceae conserved orthologous set (RosCOS) sequences (Cabrera et al. 2009). The recent release of draft genome sequences for three Rosaceae genera [woodland strawberry 'Hawaii 4' (*F. vesca* ssp. *vesca*) (Shulaev et al. 2011), 'Golden Delicious' apple (*Malus* × *domestica* Borkh.) (Velasco et al. 2010), and peach doubled haploid 'Lovell' (*Prunus persica*) (Sosinski et al. 2010)], along with the availability of reference maps for *Prunus* and *Fragaria*, as well as RosCOS-derived markers, have provided tools that have allowed the discovery of regions of conserved genomic synteny. These discoveries have led to the proposal of a common ancestor for the family with a genome structure of nine chromosomes (Velasco et al. 2010; Vilanova et al. 2008).

Several genetic maps have been constructed for red raspberry using a variety of marker types, including amplified fragment length polymorphic (AFLP), genomic simple sequence repeat (SSR), expressed sequence tag-SSR (EST-SSR), and gene-based markers (Graham et al. 2004, 2006; Sargent et al. 2007; Woodhead et al. 2008, 2010). The first published genetic linkage map of *Rubus* was developed using AFLP and SSR markers in progeny from an intraspecific cross between elite cultivars of red raspberry 'Latham' and 'Glen Moy'. This map of nine linkage groups (LG) consists of 273 markers, including 30 SSR and four EST-SSR (Graham et al. 2004). Quantitative loci for two morphological traits—variation in extent of spines, and root sucker density and spread from mother plant—were genetically mapped. However, since two of the LG are composed of non-transferable AFLP markers only, the map is not useful for comparative analyses between genera.

Subsequently, the 'Latham' × 'Glen Moy' genetic linkage map was updated, and the population used to genetically map gene *H* controlling cane pubescence and associated disease resistance to some common *Rubus* fungal pathogens (Graham et al. 2006). Although the addition of 20 SSR markers reduced the number of LG from 9 to 8, AFLP is still the predominant marker type. EST libraries from root tissue derived from 'Latham' and meristematic bud tissues derived from 'Glen Ample' have recently been generated (Woodhead et al. 2008). These libraries were mined to identify EST-SSR (Woodhead et al. 2008) and to develop functional gene-based markers (Woodhead et al. 2010). The newly developed markers were placed on the 'Latham' × 'Glen Moy' linkage map, bringing the total number of gene-based markers to 97 on 7 LG, including 37 orthologous gene-based markers from *Prunus* Group 6 (G6).

A second genetic linkage map that consists of seven LG constructed of 95 AFLP, 22 SSR, and 2 gene-based markers, was developed from another intra-specific cross between elite cultivars of red raspberry 'Malling Jewel' and 'Malling Orion' (Sargent et al. 2007). While this map is considered saturated, the high number of AFLP markers limits marker transferability.

The mapping of gene-based markers from *Prunus* G6 on to *Rubus* LG2 demonstrated by Woodhead et al. (2010), and the more efficient inter-generic transferability of EST-SSR relative to genomic SSR markers (Lewers et al. 2005; Zorrilla-Fontanesi et al. 2011) suggests that orthologous markers are key to successful comparative genomic analyses in Rosaceae.

Two recent studies on transferability of *Fragaria*-derived markers to *Rubus* and *Rosa* illustrate the importance of marker source for successful transfer between Rosaceae genera. Lewers et al. (2005) assessed the transferability of GenBank-derived *Fragaria* EST-SSR to be 32% to blackberry, and 20% to raspberry. In the same study, the transferability of primers from GenBank-derived *Fragaria* genomic SSR was lower, at 26% in blackberry, and 18% in raspberry. Zorrilla-Fontanesi et al. (2011) reported that primer pairs developed from *Fragaria* EST-SSR successfully amplified a product 20% of the time in *Rubus*, and 29% of the time in *Rosa*. In the same study, primer pairs developed from *Fragaria* genomic SSR successfully amplified a product 16% of the time in *Rubus*, and 19% of the time in *Rosa*. In general, these results indicate that the transferability of EST-SSR markers is higher than that of genomic SSR markers, though neither marker type is especially efficient; therefore additional PCR-based markers are needed to allow for the comparison of the *Rubus* genome with other members of Rosaceae.

The aim of this work was to construct genetic linkage maps of black and red raspberry based on an inter-specific progeny and enriched in orthologous molecular markers. This genetic map will be the first comprised entirely of molecular markers designed from sequenced DNA, as opposed to markers derived from random DNA. The number of orthologous, gene-based markers will augment the existing number of transferable markers to facilitate the assessment of syntenic relationships of raspberry with strawberry, apple and peach. Comparative genomic studies among these economically important genera will provide new insights into the degree of genetic conservation at the family level. This will in turn provide new tools for Rosaceae crop geneticists and breeders, especially for breeders of less well-studied Rosaceae crops and ornamentals.

## Methods

### Plant material and DNA extraction

An inter-specific cross between black raspberry *R. occidentalis* 96395S1 (S1) and red raspberry *R. idaeus* 'Latham' made in 2005 resulted in 500 $F_1$ progeny (labeled S1×Latham) that were planted during 2005 at Plant & Food Research, Motueka, New Zealand. S1 lacks spines and has dark purple fruit, while 'Latham' has spines and red fruit. Pedigrees for the parents are illustrated in Fig. 2. The genetic mapping population is comprised of 155 individuals selected from the first 200 plants in the planting

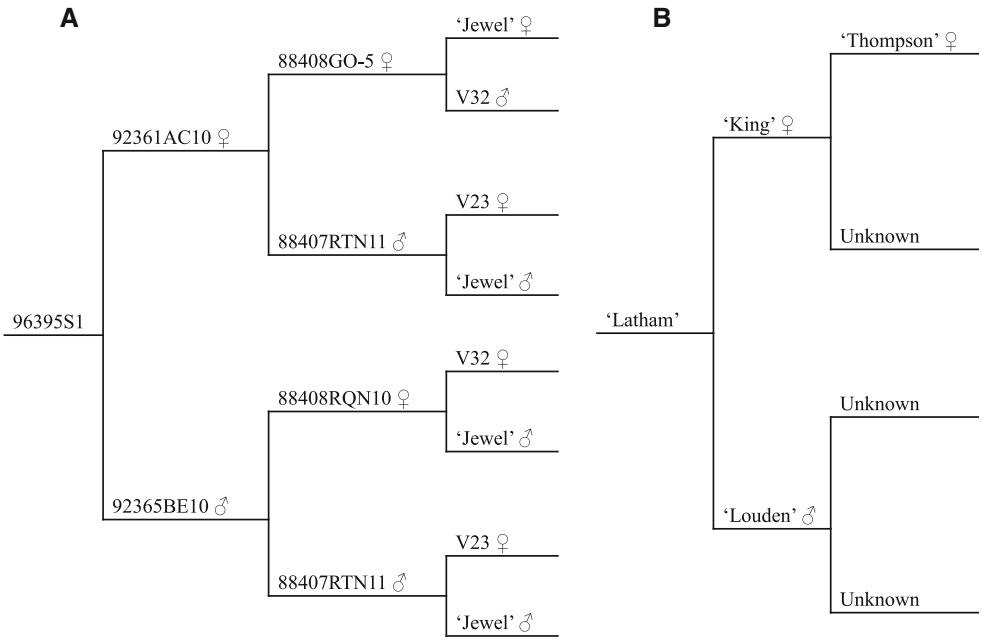



**Fig. 2** Parental pedigrees. **a** Partial pedigree of black raspberry parent 96395S1 (*Rubus occidentalis*). Great grandparents V32 and V23 are full siblings. Grandparents 88408GO-5 and 88408RQN10 are full siblings; *R* indicates the individual was produced by reciprocal cross. Individuals used in the crosses 88408 and 88407 are half-siblings as they are both derived from 'Jewel'. Parents 92361AC10 and 92365BE10 share the same pollen donor (88407RTN11). **b** Known pedigree of red raspberry parent 'Latham' (*Rubus idaeus*)

block (EA501–EA701) on the basis of production of sufficient fruit for analysis of polyphenolic content. DNA was extracted from young leaves using a modified CTAB method (Kobayashi et al. 1998).

## Genetic markers

Prior to screening the 155 progeny used for linkage analysis, an initial set of 269 primer pairs covering 250 loci were pre-screened for amplification of a polymorphic product over the mapping parents and a subset of 14 $F_1$ individuals using the high-resolution melting (HRM) technique (Wittwer et al. 2003). The 269 primer pairs were comprised of 194 published *Rubus* primer pairs (Amsellem et al. 2001; Castillo et al. 2010; Graham et al. 2004, 2006; Lewers et al. 2008; Lopes et al. 2006; Sargent et al. 2009; Woodhead et al. 2008, 2010; Zorrilla-Fontanesi et al. 2011) (28 of which did not include amplicon sequence or GenBank accession information), 45 RosCOS primer pairs (Cabrera et al. 2009), and 30 previously unpublished primer pairs from *Prunus persica* (peach) and *Rubus* sp. (Set A) (Table 1). A subset of 45 primer pairs, including 11 RosCOS, that did not amplify a product under the conditions used for HRM, were amplified under polymerase chain reaction (PCR) conditions used for direct PCR product sequencing and analyzed for amplicon size with agarose gel electrophoresis. Primer pairs that amplified a single PCR product in both parents were sequenced in both directions using BigDye Terminator v. 3.1 (BigDye™ Terminator Version 3.1 Ready Reaction Cycle Sequencing Kit, Applied Biosystems, Inc., Carlsbad, CA). The sequenced fragments were scanned for SNP using Sequencher™ 4.5 (demo version, Ann Arbor, MI) and new locus-specific primer pairs compatible with HRM were designed. As the pre-released *Rubus* genome sequence became available (J. Udall, personal communication), this was used to redesign the primer sequences for use with HRM of 195 published markers covering 72 loci (Set B), and to design 754 new HRM-based *Rubus*-specific primer pairs covering 198 loci (Set C) (Table 1). Sequences from *Arabidopsis thaliana*, *Cucumis melo*, *Fragaria* sp., *Malus* × *domestica*, *Prunus persica*, *Rubus* sp., *Solanum lycopersicum*, and Rosaceae conserved orthologous set, were searched using BLAST in the pre-released *Rubus* genome (Table 1; Supplemental Tables 1, 2). PCR primers were then designed using Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) (Bushakra et al. 2012).

## PCR and HRM conditions

HRM is a high-throughput, closed-tube melting curve analysis that uses a high-affinity double-stranded DNA-binding fluorescent dye to discriminate between genotypes relative to the presence or absence of a SNP (Reed and Wittwer 2004; Wittwer et al. 2003) or small insertion-deletion (indel) (Montgomery et al. 2007). The sensitivity of resolution, through-put capacity, and ease of use of HRM make this a useful technique for genotyping. PCR prior to HRM analyses were performed in a Roche LightCycler® 480 (Foster City, CA) in volumes of either 7 µl (384-well plates) or 10 µl (96-well plates) as in Bushakra et al. (2012). The PCR cycles were followed by HRM analysis as in Chagné et al. (2008). PCR for direct sequencing and amplicon visualizations were carried out as in Bushakra et al. (2012).

## Data collection and linkage analysis

Primer pairs that amplified a polymorphic product in the mapping population were scored based on the differences in HRM melting curves (Wittwer et al. 2003). JoinMap® v3.0 (van Ooijen and Voorrips 2001) software was used to construct the linkage map using the double pseudo-test-cross mapping strategy (Grattapaglia and Sederoff 1994). The LOD threshold for grouping was five and the Kosambi mapping function was used to convert recombination units into genetic distances.

## Genetic map comparison to published Rosaceae genomes

Sequences orthologous to the gene-based *Rubus* markers were searched in the genomes of *Fragaria vesca* (Shulaev et al. 2011), *Malus* (Velasco et al. 2010), and *Prunus* (Sosinski et al. 2010) using BLAST with a default expected value of $E^{-10}$ (Altschul et al. 1990). The physical locations of the orthologous regions in the queried genomes were used to construct physical maps for *F. vesca*, *Malus*, and *Prunus* using MapChart© (Voorrips 2002). The physical map of each genus was aligned independently to the S1 and 'Latham' linkage maps using the orthologous markers in common. Percentage of map similarity between the 'Latham' parental map and the physical maps for *F. vesca*, *Malus*, and *Prunus* was determined by calculating the distance between the top- and bottom-most markers in common. The BLAST-generated physical maps were compared with the published reference genetic maps for FV×FB (FLGI–FLGVII) and T×E (G1–G8) (www.rosaceae.org) to check the accuracy of the BLAST-derived map.

## Ancestral chromosome contribution determination

To provide an estimate of the ancestral chromosome contribution to and proportion of each *Rubus* LG, the orthologous markers genetically mapped in S1×Latham were

**Table 1** Results and sources of primer sequences used for marker development and molecular mapping in *Rubus occidentalis* 96395S1 × *R. idaeus* 'Latham' progeny

| Type of sequence | Source of sequence | Primer pairs tested | Loci covered | Loci mapped | Markers polymorphic, not mapped | Markers monomorphic | Markers no amplification | Markers complex pattern | Primers not used, alternative primer sequence | Amplification success (%)[a] | Mapping success (%)[b] | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Set A: initial primer pairs screened | | | | | | | | | | | | |
| DNA | Fsp | 15 | 6 | 2 | 1 | 1 | 5 | 5 | 1 | 64 | 22 | Sargent et al. (2007) |
| DNA | Fsp | 9 | 9 | | | 1 | 6 | 2 | | 33 | 0 | Zorrilla-Fontanesi et al. (2011) |
| DNA | M×d | 1 | 1 | | | 1 | | | | 100 | 0 | Sargent et al. (2007) |
| DNA | Ra | 2 | 2 | | | | 2 | | | 0 | 0 | Amsellem et al. (2001) |
| DNA | Rh | 3 | 3 | | | 2 | | 1 | | 100 | 0 | Castillo et al. (2010) |
| DNA | Rh | 10 | 10 | | | 4 | 4 | 2 | | 60 | 0 | Lopes et al. (2006) |
| DNA | Ri | 1 | 1 | | | 1 | | | | 100 | 0 | Castillo et al. (2010) |
| DNA | Ri | 5 | 2 | 2 | | 1 | 2 | | | 60 | 67 | Previously unpublished |
| DNA | RL | 50 | 50 | 20 | 4 | 10 | 9 | 7 | | 86 | 47 | Graham et al. (2004) |
| DNA | RL | 35 | 35 | 1 | 4 | 8 | 15 | 7 | | 57 | 5 | Graham et al. (2006) |
| DNA | RL | 1 | 1 | | | | 1 | | | 0 | 0 | Zorrilla-Fontanesi et al. (2011) |
| Sub-total | | 132 | 120 | 25 | 9 | 29 | 42 | 26 | 1 | | | |
| EST | Fsp | 2 | 1 | 1 | | | 1 | | | 50 | 100 | Zorrilla-Fontanesi et al. (2011) |
| EST | Pp | 22 | 22 | 1 | 1 | | 13 | 7 | | 41 | 11 | Previously unpublished |
| EST | RCOS | 45 | 45 | 1 | 1 | 8 | 24 | 11 | | 47 | 5 | Cabrera et al. (2009) |
| EST | Rh | 20 | 20 | 4 | 1 | 2 | 12 | 1 | | 40 | 50 | Lewers et al. (2008) |
| EST | Ri | 3 | 1 | 1 | | 1 | 1 | | | 67 | 50 | Previously unpublished |

**Table 1** continued

| Type of sequence | Source of sequence | Primer pairs tested | Loci covered | Loci mapped | Markers polymorphic, not mapped | Markers monomorphic | Markers no amplification | Markers complex pattern | Primers not used, alternative primer sequence | Amplification success (%)[a] | Mapping success (%)[b] | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EST | RL | 2 | 2 | | | 2 | | | | 100 | 0 | GDR |
| EST | RL | 7 | 7 | 2 | 1 | 2 | 2 | | | 71 | 40 | Graham et al. (2004) |
| EST | RL | 25 | 25 | 8 | 1 | 7 | 6 | 3 | | 76 | 42 | Woodhead et al. (2008) |
| mRNA | Fsp | 11 | 7 | 1 | | 1 | 8 | 1 | | 27 | 33 | Sargent et al. (2007) |
| Sub-total | | 137 | 130 | 19 | 5 | 23 | 67 | 23 | 0 | | | |
| Total | | 269 | 250 | 44 | 14 | 52 | 109 | 49 | 1 | | | |
| Set B: primers redesigned | | | | | | | | | | | | |
| DNA | Ra | 2 | 2 | 1 | | 1 | | | | 100 | 50 | Amsellem et al. (2001) |
| EST | RL | 8 | 8 | 3 | | 4 | | 1 | | 0 | 0 | GDR |
| DNA | Ri | 2 | 1 | | | 2 | | | | 100 | 0 | Graham et al. (2004) |
| EST | RCOS | 100 | 22 | 14 | 1 | 44 | 19 | 11 | 11 | 79 | 20 | Cabrera et al. (2009) |
| EST | Ri | 7 | 7 | 3 | | 1 | 3 | | | 57 | 75 | Woodhead et al. (2008) |
| EST | Ri | 76 | 32 | 16 | 4 | 22 | 4 | 25 | 5 | 94 | 24 | Woodhead et al. (2010) |
| Total | | 195 | 72 | 37 | 5 | 74 | 26 | 37 | 16 | | | |
| Set C: primers newly designed | | | | | | | | | | | | |
| DNA | M×d, Pp, Ri, Fsp | 41 | 13 | 6 | 1 | 15 | 9 | 4 | 6 | 74 | 23 | |
| EST, cDNA, mRNA, protein | At, M×d, Ri, Ro, Rsp, Fsp | 166 | 75 | 27 | 2 | 94 | 19 | 12 | 12 | 88 | 20 | |
| EST, cDNA, mRNA | At, Sl, Cm, Rsp | 496 | 89 | 22 | 1 | 250 | 98 | 67 | 58 | 78 | 6 | |
| DNA | At, Sl, Cm | 51 | 21 | 0 | 3 | 20 | 18 | 4 | 6 | 60 | 0 | |
| Total | | 754 | 198 | 55 | 7 | 379 | 144 | 87 | 82 | | | |
| Grand Totals | | 1,218 | 520 | 136 | 26 | 505 | 279 | 173 | 99 | | | |

**Table 1** continued

| Type of sequence | Source of sequence | Primer pairs tested | Loci covered | Loci mapped | Markers polymorphic, not mapped | Markers monomorphic | Markers no amplification | Markers complex pattern | Primers not used, alternative primer sequence | Amplification success (%)[a] | Mapping success (%)[b] | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 939 markers amplified | | 77.1% | | 14.5% | | 53.8% | | 18.4% | | | | |

[a] Calculated as the number of primer pairs that amplified a product, divided by the number of primer pairs tested, minus duplicates

[b] Calculated as the number of mapped loci, divided by the number of primer pairs that amplified a product, minus duplicates

At, *Arabidopsis thaliana*; Cm, *Cucumis melo*; RCOS, Rosaceae conserved orthologous set; Fsp, *Fragaria* sp.; F×a, *Fragaria* × *ananassa*; M×d, *Malus* × *domestica*; Pp, *Prunus persica*; Rsp, *Rubus* sp.; Ra, *Rubus alceifolius*; Rh, *Rubus hybrida*; Ri, *Rubus idaeus*; RL, 4*n Rubus* L.; Ro, *Rubus occidentalis*; Sl, *Solanum lycopersicum*

compared with the orthologous sequence positions, as determined by BLAST analysis, in the genomes of *F. vesca*, *Malus*, and *Prunus*. Markers in common were used to align the 'Latham' genetic linkage map to the BLAST-generated physical maps of *F. vesca*, *Malus*, and *Prunus*, and with the published ancestral representations (Illa et al. 2011; Vilanova et al. 2008).

## Results

### *Rubus* genetic linkage map construction

In total, 1,218 primer pairs were evaluated in the S1× Latham population (Table 1). Of the 269 initial primer pairs including the 45 whose products were directly sequenced (Set A), 44 (16%) amplified a product that was genetically mapped; 14 (5%) amplified a polymorphic product that was not mapped; 52 (19%) produced a monomorphic amplicon, 109 (40.5%) did not amplify a PCR product; 49 (18%) exhibited complex or unclear melting curves in the progeny screening set, and one pair was not used because it was an alternative primer sequence for a mapped marker.

Of the 195 primer pairs that were redesigned using the *Rubus* genome sequence (Set B), 37 (19%) amplified a product that was genetically mapped; 5 (2.5%) amplified a polymorphic product that was not mapped; 74 (38%) produced a monomorphic amplicon, 26 (13%) did not amplify a PCR product, and 37 (19%) exhibited complex or unclear melting curves in the progeny screening set, and 16 (8%) were not used because they were alternative primer sequences for mapped markers.

Of the 754 newly designed primer pairs (Set C), 123 (16.3%) produced amplicons that were determined to be polymorphic and screened over the set of 155 progeny; 379 primer pairs (50.3%) produced a monomorphic amplicon, 144 (19%) did not amplify a PCR product, and 87 primer pairs (11.5%) produced amplicons that exhibited complex or unclear results in the progeny screening set. Of the 123 polymorphic amplicons, 55 (45%) were genetically mapped. In summary, of the total 754 primer pairs designed, 55 (7%) were genetically mapped.

The linkage groups illustrated in Fig. 3 are labeled with the original linkage group (OLG) number as assigned by Graham et al. (2004) shown first in parentheses, followed by the proposed *Rubus* linkage group (RLG) number to place the RLG in the same order as the published FV×FB LG. Of the 136 mapped markers, 131 were placed on at least 1 of the 2 parental maps. The remaining five markers were removed because of skewed segregation ratios. The parental genetic map for 96395S1 consists of 29 markers, 17 of which are unique to S1, averaging one marker every 10 cM, and covering 306 cM over 6 LG. S1 had only one heterozygous

marker (Ro_1K15) in RLG1. The parental genetic map for 'Latham' consists of 114 markers, 101 of which are unique to 'Latham', averaging 1 marker every 5 cM, and covering 561 cM over 7 LG with RLG7 in 2 parts. The 2 parents share 13 markers (Fig. 3; Table 2; Supplemental Table 1). The 'Latham' genetic linkage map was used for comparative figures with *Fragaria*, *Malus*, and *Prunus* since it is the more complete map of the two parents. Consensus LG for S1 and 'Latham' could not be constructed due to a lack of markers in common between the parents.

## Comparative genome mapping between *Rubus* and *Fragaria*

The comparison of the 131 markers that comprise the linkage maps of 96395S1 and 'Latham' with the draft genome assembly of *F. vesca* using BLAST analysis of marker sequences identified 90 markers in common between the two genera and demonstrates a nearly 1:1 LG relationship (Figs. 4, 5; Supplemental Fig. 1). BLAST expected values (*E* value) ranged from $2.00E^{-06}$ with 92% identity, to $0.00E^{+00}$ with 99% identity, with only five markers having *E* values $>E^{-15}$ (Table 3; Supplemental Fig. 1; Supplemental Table 2). Based on the positions of 77 markers common to both genera, each of the 7 *Rubus* LG could be aligned to 1 of the 7 *Fragaria* chromosomes, ranging from 2 markers on RLG4, to 12 markers on RLG3, and covering 96% (538 of 561 cM) of the 'Latham' genetic linkage map (Fig. 4; Supplemental Fig. 1). Colinearity was largely conserved between *Rubus* and *Fragaria* although a few differences were observed in gene order. Of the 90 markers in common, 5 (EMF×aCAD1B, RiSNF4, RiPPC1, RubARSFL_33_Exp3 and EMF×aACO1B) had significant BLAST scores on 2 different LG; and 15 (16.6%) did not map to the homologous LG (Fig. 3). These 15 markers were designed from candidate gene sequence (*RiMYB10*, *RiCXE*, *RiSNF4*, *RiPKP2*, *RiInvAlkE*, *RiADH*, *RiHXK*, *RiFRUCT4*, *RiPAL2* and *RiFRK*), *Rubus* SSR (Ro_1K15 and Ro_1E22), and *Rubus* EST (Ru_EE284365 and ERubLR_SQ005-3_H01). Marker Ri_GDSNP00321 was designed from a 'Golden Delicious' single nucleotide polymorphism (GDSNP) sequence.

## Comparative genome mapping between *Rubus* and *Malus*

The comparison of the 131 markers that comprise the linkage maps of 96395S1 and 'Latham' with the draft genome assembly of *Malus* using BLAST analysis of marker sequences identified 80 markers in common between the 2 genera, with *E* values ranging from $5.00E^{-05}$ with 83% identity, to $0.00E^{+00}$ with 99% identity, with only 5 markers having *E* values $>E^{-15}$ (Table 3; Supplemental Fig. 2; Supplemental Table 2). Based on the positions of 70 markers (including the three GDSNP-derived markers) common to both genera, each of the 7 *Rubus* LG could be aligned with 1, 2 or 3 segments of the 11 homeologous chromosomes in *Malus* (Velasco et al. 2010), ranging from 1 marker on RLG4, to 15 markers on RLG3, and covering 77% (430 of 561 cM) of the 'Latham' genetic linkage map (Fig. 4; Supplemental Fig. 2). Of the 80 markers in common, 41 (51%), including EMF×aACO1B, have significant BLAST scores on homeologous chromosomes; 6 markers (7.5%) (RiF3′H, RubARSFL_33_Exp3, RubARSFL_35_Cel2, EMF×aACO1B, Ri_5O21 and RiRosCOS1281) have significant BLAST scores on non-homeologous chromosomes; and 39 markers (47%) occur once only. Of the 80 markers in common, 19 (24%) did not map to the homologous LG (Fig. 3). These 19 markers are from *Rubus* EST (ERubLR_SQ05-3_E02), various genes (*RiSNF4*, *RiHCT/HQT*, *RiSNF2*, *RiInvAlkA*, *RiMYB*, *RiHXK*, *Ri4Co1*, *RiSAMD*, *RiLTP*, *RiG6PD*, *RiFRUCT4*, *RiPAL2* and *RibHLH*), *Fragaria* gene (*EMF×aCAD1B*), and RosCOS (RiRosCOS3524, RiRosCOS2381, RiRosCOS1412, RiRosCOS1360).

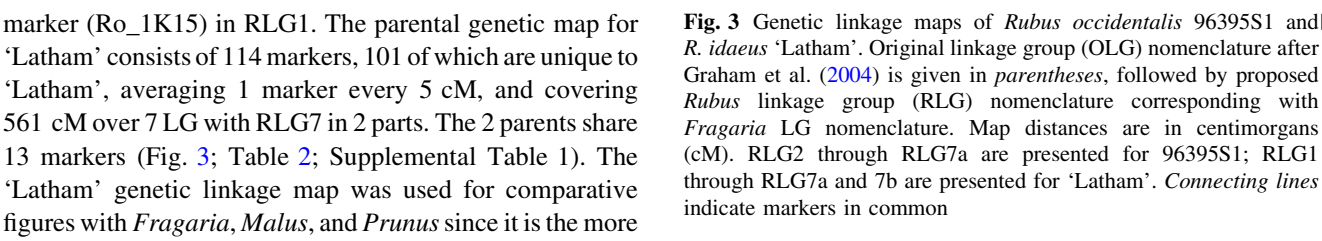
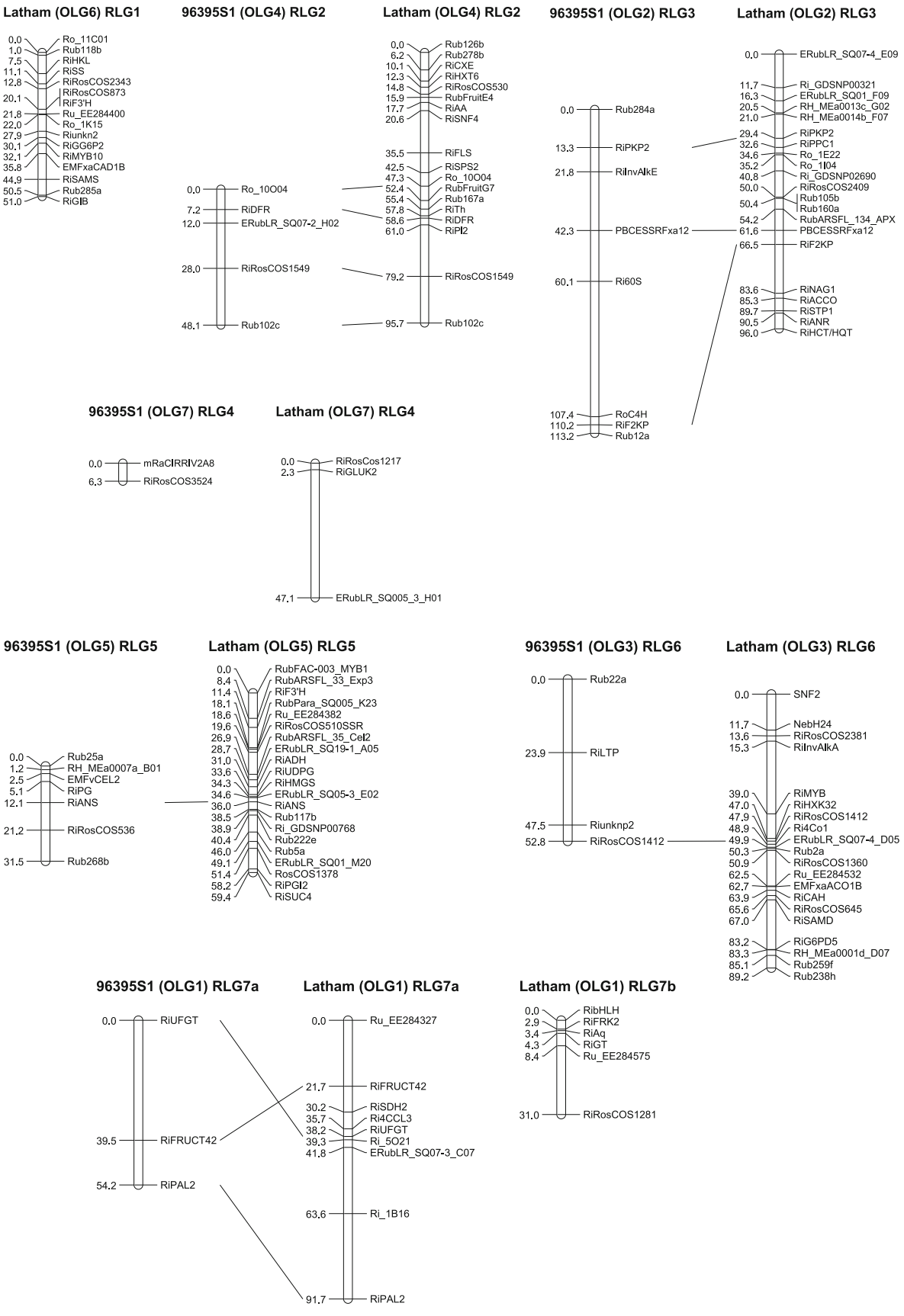


**Fig. 3** Genetic linkage maps of *Rubus occidentalis* 96395S1 and *R. idaeus* 'Latham'. Original linkage group (OLG) nomenclature after Graham et al. (2004) is given in *parentheses*, followed by proposed *Rubus* linkage group (RLG) nomenclature corresponding with *Fragaria* LG nomenclature. Map distances are in centimorgans (cM). RLG2 through RLG7a are presented for 96395S1; RLG1 through RLG7a and 7b are presented for 'Latham'. *Connecting lines* indicate markers in common

## Comparative genome mapping between *Rubus* and *Prunus*

The comparison of the 131 markers that comprise the linkage maps of 96395S1 and 'Latham' with the draft genome assembly of *Prunus* using BLAST analysis of marker sequences identified 86 markers in common between the 2 genera, with *E* values ranging from $2.00E^{-06}$ with 85% identity, to $0.00E^{+00}$ with 100% identity, with only 5 markers having *E* values $>E^{-15}$ (Table 3; Supplemental Fig. 3; Supplemental Table 2). Based on the positions of 86 markers common to both genera, each of the seven *Rubus* LG could be aligned with 1, 2 or 3 segments of the *Prunus* chromosomes, ranging from 3 markers on RLG4, to 14 markers on RLG3, and covering 90% (505 of 561 cM) of the 'Latham' genetic linkage map (Fig. 4; Supplemental Fig. 3). Of the 86 markers in common, 5 (6%) (RiMYB10, RiPPC1, EMF×aACO1B, RiSDH2 and RiPAL2) had significant BLAST scores on 2 different LG; and 15 (17%) did not map to the homologous LG (Fig. 3). These 15 markers are from *Rubus* SSR (Ro_1K15), *Fragaria* gene (*EMF×aCAD1B*), various genes (*RiSNF4*, *RiInvAlkE*, *RiHCT*/HQT, *SNF2*, *RiInvAlkA*, *RiMYB*, *Ri4Co1*,


Latham (OLG6) RLG1
0.0 Ro_11C01
1.0 Rub118b
7.5 RiHKL
11.1 RiSS
12.8 RiRosCOS2343
20.1 RiRosCOS873
RiF3'H
21.8 Ru_EE284400
22.0 Ro_1K15
27.9 Riunkn2
30.1 RiGG6P2
32.1 RiMYB10
35.8 EMFxaCAD1B
44.9 RiSAMS
50.5 Rub285a
51.0 RiGIB
96395S1 (OLG4) RLG2
0.0 Ro_10O04
7.2 RiDFR
12.0 ERubLR_SQ07-2_H02
28.0 RiRosCOS1549
48.1 Rub102c
Latham (OLG4) RLG2
0.0 Rub126b
6.2 Rub278b
10.1 RiCXE
12.3 RiHXT6
14.8 RiRosCOS530
15.9 RubFruitE4
17.7 RiAA
20.6 RiSNF4
35.5 RiFLS
42.5 RiSPS2
47.3 Ro_10O04
52.4 RubFruitG7
55.4 Rub167a
57.8 RiTh
58.6 RiDFR
61.0 RiPI2
79.2 RiRosCOS1549
95.7 Rub102c
96395S1 (OLG2) RLG3
0.0 Rub284a
13.3 RiPKP2
21.8 RiInvAlkE
42.3 PBCESSRFxa12
60.1 Ri60S
107.4 RoC4H
110.2 RiF2KP
113.2 Rub12a
Latham (OLG2) RLG3
0.0 ERubLR_SQ07-4_E09
11.7 Ri_GDSNP00321
16.3 ERubLR_SQ01_F09
20.5 RH_MEa0013c_G02
21.0 RH_MEa0014b_F07
29.4 RiPKP2
32.6 RiPPC1
34.6 Ro_1E22
35.2 Ro_1I04
40.8 Ri_GDSNP02690
50.0 RiRosCOS2409
50.4 Rub105b
Rub160a
54.2 RubARSFL_134_APX
61.6 PBCESSRFxa12
66.5 RiF2KP
83.6 RiNAG1
85.3 RiACCO
89.7 RiSTP1
90.5 RiANR
96.0 RiHCT/HQT
96395S1 (OLG7) RLG4
0.0 mRaCIRRIV2A8
6.3 RiRosCOS3524
Latham (OLG7) RLG4
0.0 RiRosCos1217
2.3 RiGLUK2
47.1 ERubLR_SQ005_3_H01
96395S1 (OLG5) RLG5
0.0 Rub25a
1.2 RH_MEa0007a_B01
2.5 EMFvCEL2
5.1 RiPG
12.1 RiANS
21.2 RiRosCOS536
31.5 Rub268b
Latham (OLG5) RLG5
0.0 RubFAC-003_MYB1
8.4 RubARSFL_33_Exp3
11.4 RiF3'H
18.1 RubPara_SQ005_K23
18.6 Ru_EE284382
19.6 RiRosCOS510SSR
26.9 RubARSFL_35_Cel2
28.7 ERubLR_SQ19-1_A05
31.0 RiADH
33.6 RiUDPG
34.3 RiHMGS
34.6 ERubLR_SQ05-3_E02
36.0 RiANS
38.5 Rub117b
38.9 Ri_GDSNP00768
40.4 Rub222e
46.0 Rub5a
49.1 ERubLR_SQ01_M20
51.4 RosCOS1378
58.2 RiPGI2
59.4 RiSUC4
96395S1 (OLG3) RLG6
0.0 Rub22a
23.9 RiLTP
47.5 Riunknp2
52.8 RiRosCOS1412
Latham (OLG3) RLG6
0.0 SNF2
11.7 NebH24
13.6 RiRosCOS2381
15.3 RiInvAlkA
39.0 RiMYB
47.0 RiHXK32
47.9 RiRosCOS1412
48.9 Ri4Co1
49.9 ERubLR_SQ07-4_D05
50.3 Rub2a
50.9 RiRosCOS1360
62.5 Ru_EE284532
62.7 EMFxaACO1B
63.9 RiCAH
65.6 RiRosCOS645
67.0 RiSAMD
83.2 RiG6PD5
83.3 RH_MEa0001d_D07
85.1 Rub259f
89.2 Rub238h
96395S1 (OLG1) RLG7a
0.0 RiUFGT
39.5 RiFRUCT42
54.2 RiPAL2
Latham (OLG1) RLG7a
0.0 Ru_EE284327
21.7 RiFRUCT42
30.2 RiSDH2
35.7 Ri4CCL3
38.2 RiUFGT
39.3 Ri_5O21
41.8 ERubLR_SQ07-3_C07
63.6 Ri_1B16
91.7 RiPAL2
Latham (OLG1) RLG7b
0.0 RibHLH
2.9 RiFRK2
3.4 RiAq
4.3 RiGT
8.4 Ru_EE284575
31.0 RiRosCOS1281

*RiSAMD*, *RiG6PD*, *RiSDH2* and *RiFRUCT4*), and RosCOS (RiRosCOS2381 and RiRosCOS1412).

## Comparative genome mapping between *Rubus* and the Rosaceae ancestral genome

The ancestral genome structure of nine chromosomes (A1–A9) and their proposed distribution among and contribution to the genomes of *Rubus* (RLG1–RLG7), *Fragaria vesca* (FLGI–FLGVII), *Malus* (MLG1–MLG17), and *Prunus* (G1–G8) are illustrated in Fig. 5. The 9 ancestral chromosomes are distributed in 14 segments across the *Rubus* genome, with 2 *Rubus* linkage groups (including both segments of RLG7) composed of 1 ancestral chromosome and the remaining 5 *Rubus* linkage groups composed of 2 or more ancestral chromosomes.

RLG1 and FLGI both have A7 as a contributor (Fig. 5). A7 also contributes to G7 (Vilanova et al. 2008), and to segments of apple homeologous chromosomes MLG2 and MLG15 (Illa et al. 2011). To obtain a consensus for ancestral contribution, a change in orientation of RLG1 is needed relative to the representation from Graham et al.'s (2004) original LG6 (Fig. 3), and also to G7. Five markers in common between RLG1 and FLGI support the contribution of A5; however, Illa et al. (2011) shows A8 to be a contributor to FLGI. The set of markers used in this study, and the results of Bushakra et al. (2012), do not demonstrate a contribution of A8 to FLGI. While there are two markers found with homology to G6, which is derived from A8, the comparison of the four genera supports A5 as the second major contributor to RLG1 and FLGI.

RLG2 and FLGII both show strong support for derivation from A1 and A2 (Fig. 5). This is in agreement with the results of Bushakra et al. (2012) and contrary to the results of Illa et al. (2011) which suggest that FLGII is derived entirely from A1. A1 also contributes to apple homeologous chromosomes MLG5 and MLG10 (Illa et al. 2011) and *Prunus* G1 and G8 (Vilanova et al. 2008). The contribution of A2 to apple homeologous chromosomes MLG8 and MLG15 (Illa et al. 2011) and *Prunus* G1 (Vilanova et al. 2008) is also supported by these data.

RLG3 and FLGIII show strong support for derivation from A8 and A9 (Fig. 5). A change in orientation of RLG3 has been made relative to the representation from Graham et al.'s (2004) original LG2 (Fig. 3). This change in orientation is also supported by the results of Bushakra et al. (2012) for apple homeologous chromosomes MLG3 and MLG11. The contributions of A8 and A9 to G4 and G6 (Vilanova et al. 2008) is also supported with these data, and a change in LG orientation brings all genera into alignment.

RLG4 and FLGIV have only three markers in common; however, the contribution of A2 is suggested for all genera (Fig. 5).

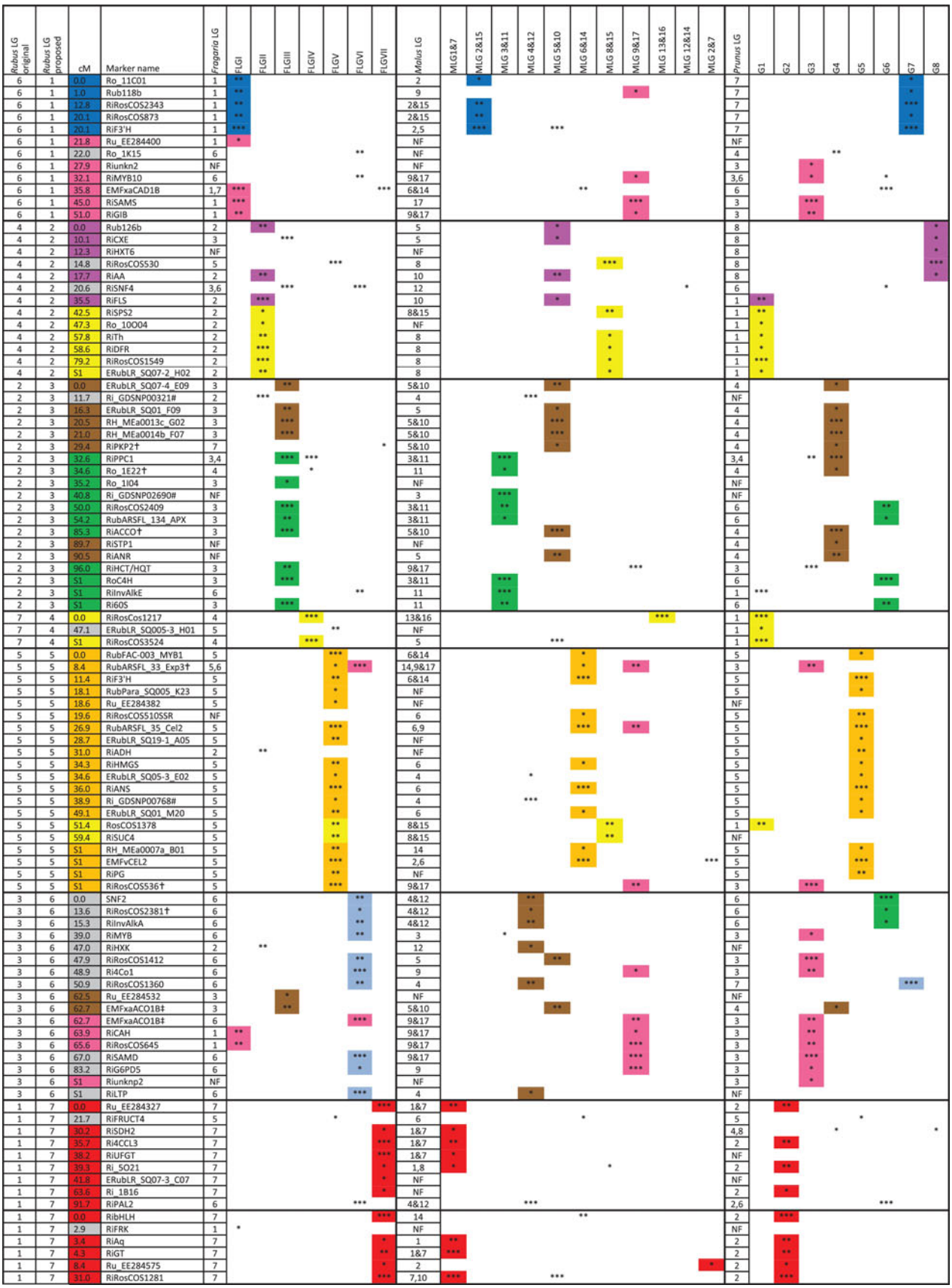
**Fig. 4** Comparison of *Rubus idaeus* 'Latham' genetic linkage map with *Fragaria*, *Malus*, and *Prunus*. Markers for which sequence was available for BLAST analysis are arranged based on the 'Latham' linkage map order; map distance is in centimorgans (cM). *Rubus* original linkage group (OLG) is based on numbering scheme by Graham et al. (2004). *Colors* indicate ancestral chromosome contribution (see Fig. 5 for color key based on Vilanova et al. 2008). Apple LG separated by "&" indicate homeologous chromosomes; those separated by a comma are non-homeologous (Velasco et al. 2010). Linkage groups separated by a comma in *Fragaria* and *Prunus* indicate markers located on non-homologous chromosomes. *Grey* regions indicate ambiguous ancestral contribution. Sequences for which similarity could not be found in the target genus are indicated as NF. Markers unique to S1 are included at the end of each LG with "S1" in place of cM values. *** *E* values of $0.00E^{+00}$ to $E^{-100}$; ** *E* values of $E^{-99}$ to $E^{-50}$; * *E* values $>E^{-50}$. ‡ The BLAST analysis for marker EMF×aACO1B shows two equally likely loci in *Fragaria*, *Malus*, and *Prunus*. Only one locus is mapped in *Rubus*. † These markers have two equally likely ancestral origins. When possible, the ancestral origin that agrees with *Fragaria* was chosen for the *Rubus* map. # These markers were designed from *Malus* 'Golden Delicious' SNP sequences (color figure online)

**Table 2** Linkage group descriptions for the parental maps of *Rubus occidentalis* 96395S1 and *R. idaeus* 'Latham'

| | Markers mapped in 96395S1 | Linkage group size (cM) | Markers mapped in 'Latham' | Linkage group size (cM) | Markers shared between parental maps[a] |
|---|---|---|---|---|---|
| RLG1 | | | 16 | 51 | 1 |
| RLG2 | 5 | 48 | 18 | 96 | 4 |
| RLG3 | 8 | 113 | 21 | 96 | 3 |
| RLG4 | 2 | 6 | 3 | 47 | 0 |
| RLG5 | 7 | 31 | 21 | 59 | 1 |
| RLG6 | 4 | 53 | 20 | 89 | 1 |
| RLG7a | 3 | 54 | 9 | 92 | 3 |
| RLG7b | | | 6 | 31 | 0 |
| Totals | 29 | 305 | 114 | 561 | 13 |

Linkage group size is in centimorgans (cM)

[a] Insufficient markers available for map integration


Rubus LG original
Rubus LG proposed
cM
Marker name
Fragaria LG
FLGI
FLGII
FLGIII
FLGIV
FLGV
FLGVI
FLGVII
Malus LG
MLG1&7
MLG 2&15
MLG 3&11
MLG 4&12
MLG 5&10
MLG 6&14
MLG 8&15
MLG 9&17
MLG 13&16
MLG 12&14
MLG 2&7
Prunus LG
G1
G2
G3
G4
G5
G6
G7
G8

RLG5, FLGV, G5 and apple homeologous chromosomes MLG6 and MLG14 show a high degree of conservation (Fig. 5). All are derived primarily from A3. There appears to be a small segment of A2 contributing to each LG and this is supported by the results of Bushakra et al. (2012). To bring RLG5 and FLGV into alignment, a change in orientation of RLG5 has been made relative to the representation from Graham et al.'s (2004) original LG5 (Fig. 3). Marker RubARSFL_33_RiExp3 appears to be derived from both A3 and A5, as this gene codes for an enzyme involved in expansin biosynthesis, and could be a member of a gene family.

RLG6 and FLGVI are interesting in that they appear to contain elements of three ancestral chromosomes A5, A6, and A9 (Fig. 5). Alignment of *Rubus* and *Fragaria* has required a change in orientation of RLG6 relative to the representation from Graham et al.'s (2004) original LG3 (Fig. 3). The contribution of A6 to FLGVI, the contribution of A5 to FLGVI and apple homeologous chromosomes MLG9 and MLG17, as well as the contribution of A9 to MLG4 and MLG12 and homeologous chromosomes MLG5 and MLG10 is supported by the results of Illa et al. (2011). The contribution of A5 to G3 is supported by the results of Vilanova et al. (2008). A change in orientation of FLGVI is suggested by Bushakra et al. (2012).

RLG7, FLGVII, G2 and apple homeologous chromosomes MLG1 and MLG7 show a high degree of conservation (Fig. 5). All are derived primarily from A4, and this single origin is supported by the results of Illa et al. (2011), Vilanova et al. (2008), and Bushakra et al. (2012).

## Discussion

### Inter-specific *Rubus* genetic map construction

We present here the first genetic linkage map of *Rubus* developed with *R. occidentalis* as a parent of the mapping progeny. Our map is composed entirely of markers derived from non-anonymous DNA sequences, the majority of which are EST-based or gene-derived. As this map was developed from an inter-specific progeny with 'Latham' as a parent, it can be aligned with existing *R. idaeus* 'Latham'-based maps (Graham et al. 2004, 2006, 2009; Kassim et al. 2009; McCallum et al. 2010; Sargent et al. 2007; Woodhead et al. 2008) to obtain a wider view of genetic diversity in *Rubus*. Overall, our map aligns with the existing maps. This new map, derived from parents with different fruit color, and enriched for orthologous markers, provides a resource for future studies on variation in fruit color and polyphenolic compounds.

Our HRM-based marker design provides a technique for high-throughput genotyping not previously utilized in *Rubus* genetic mapping studies. The direct sequencing of 45 PCR products demonstrated the ability of HRM to distinguish accurately between homoduplex and heteroduplex PCR products, providing confidence in determining the parental source of alleles. Our use of existing EST together with the pre-released genome sequence resulted in development of new orthologous markers that will be useful as anchor loci for the on-going assembly of the *Rubus* genome.

The 'Latham' genetic linkage map only was used for the comparative analyses because of the much higher number of heterozygous markers between 'Latham' (114 mapped markers) compared to 96395S1 (29 mapped markers). The difference in number of heterozygous loci in the S1 parental map compared to the 'Latham' map is likely to be the result of the degree of inbreeding in the S1 parent. Using methods described by Falconer and Mackay (1996), we calculated the inbreeding coefficient for S1 to be 0.35, indicating that 35% of alleles at a locus are identical by descent, resulting in a sparsely populated S1 genetic map. The degree of inbreeding in S1 is higher than the average of 21% reported for raspberry cultivars (Dale et al. 1993), and the 0–25% reported in both blackberry (*Rubus* subgenus *Rubus*) (Stafne and Clark 2004) and almond (*Prunus dulcis*) cultivars (Lansari et al. 1994). The parent 96395S1 has a pedigree dominated by black raspberry (*R. occidentalis*); however it was derived from crosses of black raspberry with red raspberry 'Burnethholm' with the intention to introgress spinelessness from this cultivar. The spineless type was achieved via selections V23 and V32 (Fig. 2a). From available pedigree information we calculated selection 96395S1 to have approximately 9.4% genetic contribution from red raspberry with the remaining 90.6% from black raspberry.

From known pedigree information (Fig. 2b) we used the methods described by (Falconer and Mackay 1996) to calculate the inbreeding coefficient of *R. idaeus* 'Latham' as 0, indicating that the 'Latham' parent is not inbred and is likely to be significantly more heterozygous than S1. The parent 'Latham' was included in this cross because it provides a link to the Graham et al. (2004) genetic map. The degree of genetic homozygosity of the S1 parent was not considered when it was selected for crossing with 'Latham'; rather, the emphasis was placed on developing a progeny with a range of fruit colors for color- and polyphenolic-based quantitative trait locus (QTL) analyses.

### Development of orthologous markers for use in *Rubus*

During the screening of published markers (Set A), we found 42 markers to be monomorphic or with both parents homozygous (aa×bb) in our population. Of these, 29 (71%) were previously reported as heterozygous in 'Latham'

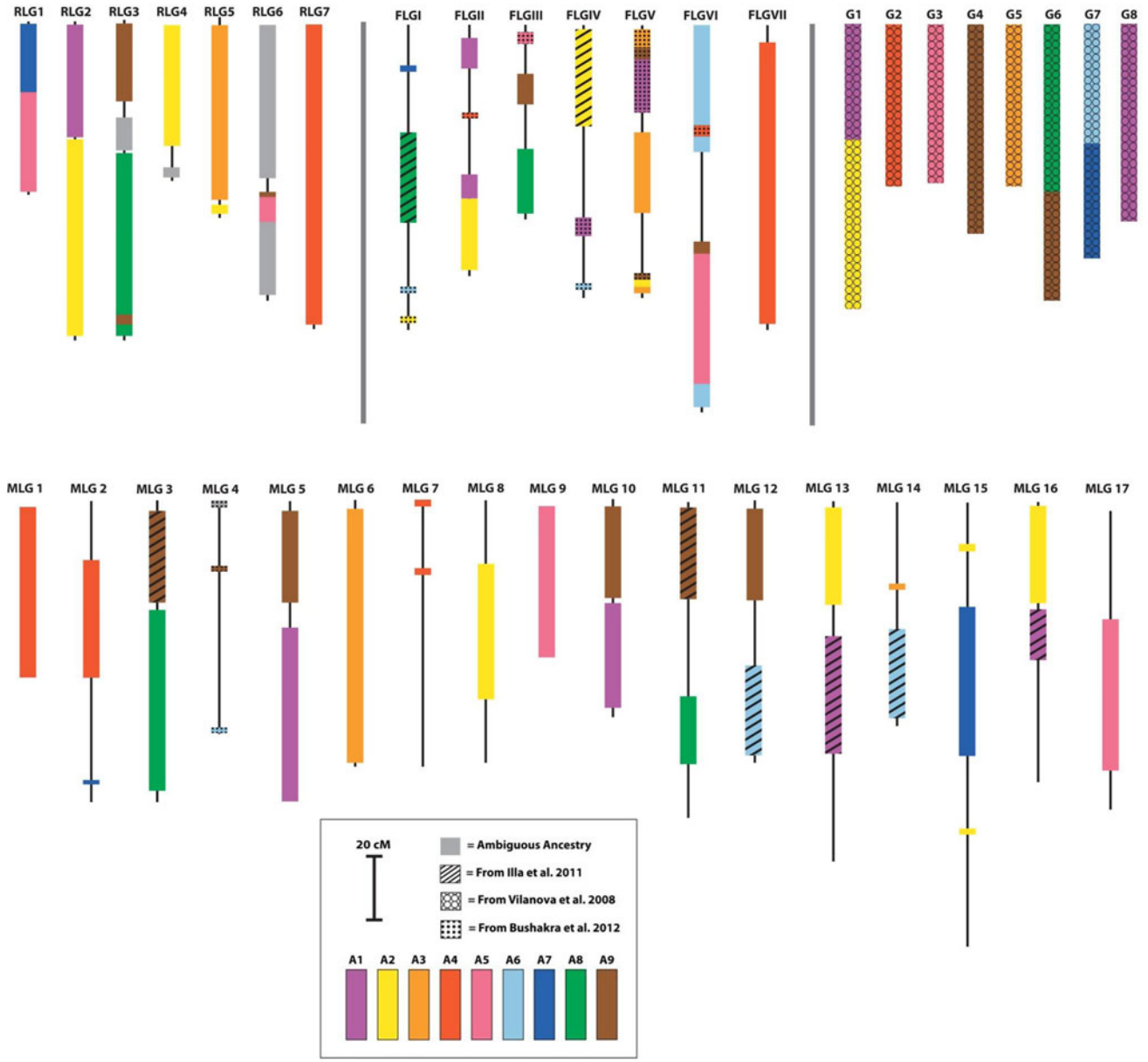



**Fig. 5** Ancestral chromosome (A1–A9) contribution to the genomes of *Rubus* linkage groups (RLG1–RLG7), *Fragaria* linkage groups (FLGI–FLGVII), *Prunus* groups (G1–G8), and *Malus* linkage groups (MLG1–MLG17). The *Rubus* LG are in order to correspond to *Fragaria*, are to scale and represent the findings of this study. The ancestral contributions for all *solid color regions* in *Fragaria* and *Malus*, and for *Prunus* [*circle pattern*, Vilanova et al. (2008)] are supported by the findings of this study. The *Fragaria* and *Malus* LG are based on information from Bushakra et al. (2012) (*dotted regions*) and Illa et al. (2011) (cross-hatched regions). For all genera: *solid black lines* indicate gaps where no markers were mapped genetically; colors follow Vilanova et al. (2008) with modifications to reflect Bushakra et al. (2012). *Grey regions* represent areas of ambiguous ancestral contribution (color figure online)

(Graham et al. 2004). As a trial screen of a subset of the published SSR markers using traditional SSR screening methods (ABI 3100 polyacrylamide gel, data not shown) indicated similar degrees of monomorphism to that exhibited following HRM analysis, we believe that this discrepancy could be caused by the use of different 'Latham' sources in the respective studies, rather than by the differences in marker analysis techniques employed. Unfortunately, synonyms for genotypes and errors in labeling and misidentifications in germplasm collections are not uncommon and spontaneous mutations and sports can arise in clonally propagated crops (Bassil et al. 2009; Gygax et al. 2004; Thompson 1995a, b). To resolve this issue, samples from different 'Latham' sources need to be genotyped. However, the variation in heterozygosity for some markers found in this study does not appear to

**Table 3** Summary of the number and occurrence of markers in common between *Rubus occidentalis* 96395S1 × *R. idaeus* 'Latham' and *Fragaria*, *Malus*, and *Prunus* genomes as determined by BLAST analyses

| | Markers in common with *Rubus*[a] | *E* value range | Markers occurring once | Markers with significant second hits[b] | Homeologous markers[b] | Aligning markers[b] | Non-homologous markers | % of non-homologous markers |
|---|---|---|---|---|---|---|---|---|
| *Fragaria* | 90 | $2E^{-06}$ to 0 | 86 | 5 | | 77 | 15 | 16.6 |
| *Malus* | 80 | $5E^{-05}$ to 0 | 39 | 6 | 41 | 70 | 19 | 23.8 |
| *Prunus* | 86 | $6E^{-13}$ to 0 | 81 | 5 | | 80 | 15 | 17.4 |

[a] Does not include the second locus for EMF×aACO1B

[b] Includes EMF×aACO1B which has significant BLAST scores on two different LG in each of the three genera, and in apple occurs on two different sets of homeologous chromosomes, yet occurs in *Rubus* once only

influence greatly the ability to compare the linkage maps, as the overall alignment of anchor loci agree among the mapping studies.

The recent studies on marker transferability by Lewers et al. (2005), Zorrilla-Fontanesi et al. (2011), and our own results, demonstrate the difficulty of identifying polymorphic loci in *Rubus*, and also illustrate that the amplification of a PCR product does not necessarily make a marker useful for map construction. In general, amplification of polymorphic loci in *Rubus* using PCR primer pairs designed from *Fragaria* sequence was more successful if the sequence was from EST (8–13%, and a total of 18 markers) than if the sequence was from genomic SSR (5–6%, and a total of 4 markers) (Table 4). Between the 2 studies, only 22 of a total of 222 markers (10%) amplified a polymorphic product in their respective *Rubus* species. In our study, 939 of 1,218 primer pairs (77%) amplified a product. Of these, 136 (14.5%) mapped, 505 (54%) were monomorphic, and 177 (18%) had complex HRM curves. The percentage of monomorphic markers in our population is similar to what was reported by Lewers et al. (2005) (40–83%) and Zorrilla-Fontanesi et al. (2011) (34–60%) and suggests that the cultivars and selections most often used for mapping studies of *Rubus* may have high gene homozygosity. Our marker design technique focused on the discovery of de novo SNP using HRM to detect sequence polymorphisms, rather than amplification of SSR repeats. As the genome sequences of more *Rubus* cultivars become available and the positions of putative SNP loci can be identified, the HRM technique will be an efficient genotyping method.

## Genetic map comparison between *Rubus* and other Rosaceae genera

The degree of genomic synteny and conservation demonstrated in this study is further evidence of the relatively close phylogenetic relationship between *Rubus* and *Fragaria* (Eriksson et al. 2003; Potter et al. 2007). These relationships are especially clear when the *Rubus* and *Fragaria* LG are oriented with each other, as they are presented in Figs. 4 and 5. This relationship leads us to propose the renumbering and reorientation of the *Rubus* LG to allow easier comparisons with *Fragaria* and to be

**Table 4** Summary of transferability of markers derived from *Fragaria* DNA sequence to raspberry and blackberry

| Source of sequence for primer design | Amplification results | Raspberry | Blackberry | Markers with potential for mapping |
|---|---|---|---|---|
| GenBank *Fragaria* EST-SSR primer pairs tested (Lewers et al. 2005) | | 28 | 23 | |
| | Amplified | 5 (18%) | 6 (26%) | |
| | Polymorphic | 3 (11%) | 3 (13%) | 6 |
| GenBank *Fragaria* genomic SSR primer pairs tested (Lewers et al. 2005) | | 20 | 19 | |
| | Amplified | 4 (20%) | 6 (26%) | |
| | Polymorphic | 1 (5%) | 1 (5%) | 2 |
| *Fragaria* EST-SSR primer pairs tested (Zorrilla-Fontanesi et al. 2011) | | 143 | NA | |
| | Amplified | 29 (20%) | | |
| | Polymorphic | 12 (8%) | | 12 |
| *Fragaria* genomic SSR primer pairs tested (Zorrilla-Fontanesi et al. 2011) | | 31 | NA | |
| | Amplified | 5 (16%) | | |
| | Polymorphic | 2 (6%) | | 2 |

*NA* not applicable

more consistent with previous work in the family, especially necessary now as the genome information for both genera increases rapidly.

Our study identified several previously undetected differences in ancestral contribution to the genomes of *Rubus* and *Fragaria* that will require further analysis (Figs. 4, 5). The data suggest that RLG 2, 3, 4, 5 and 7 and the corresponding *Fragaria* LG have retained a high degree of synteny. It appears that RLG1 varies from *Fragaria* in its ancestral makeup and that RLG6 and FLGVI both differ in their ancestral makeup relative to *Malus* and *Prunus*. Comparison of the four genera is facilitated by the high DNA sequence transferability, as the sequences of 73 (56%) of the mapped markers were identified in all four genera, and provide new insights into family-wide orthology. For example, the high degree of colinearity identified between RLG7 compared with FLGVII, G2 and MLG2 suggests that these LG have remained relatively unchanged from the ancestral state (A4). Ancestral conservation is also suggested by the level of colinearity seen between RLG5 compared with both G5 and MLG6 with respect to A3. Additional insights come from markers that mapped to non-homologous LG, or with significant BLAST scores on two different LG in *F. vesca* and *Prunus*, and those in *Malus* that are located on non-homeologous chromosomes. These markers could represent paralogous loci or be simply the best BLAST hit based on the state of assembly of the *Rubus* genome at the time of the analysis (Jan.–Apr. 2011). Genes used to design the *Rubus*-specific markers are known to be from several metabolic pathways in other plant species; therefore, some are likely to be members of gene families and not necessarily single copies. Additionally, as BLAST analysis identifies regions of similarity, but not the functionality of the region, some of these markers may be duplicates but non-functional. The variation in marker order in the genetic maps of 96395S1 and 'Latham' compared with the BLAST-derived map of *Fragaria* could indicate the amplification of other members of the gene families or the presence of paralogous genes. The two occurrences of RiRosCOS1281 on non-homeologous chromosomes (MLG7 and MLG10) and the position of RiRosCOS530 on FLGV rather than FLGII could be examples of errors in genotyping or perhaps in the draft *Malus* and *Fragaria* genome assemblies, respectively. These ambiguities will become clearer as more markers are mapped and the genome assemblies are further developed. Two of the three markers designed from *Malus* GDSNP sequences, Ri_GDSNP02690 and Ri_GDSNP00768 map to homologous LG in *Rubus*; however, these could easily be coincidental similarities rather than true homologies.

The orthologous markers developed in this study can be used to explore the genomes of other less-well characterized Rosaceae species, such as ornamentals and members of the subfamily Dryadoideae. Genomic analysis of members of Dryadoideae will lead to a more complete picture of the family and its evolution.

## Conclusions

The genetic maps of *Rubus* constructed in this study map provide a useful set of transferable and orthologous markers for comparative mapping, and a solid foundation for further studies such as quantitative trait analyses. Additionally, the maps illustrate the nearly one-to-one alignment of the linkage groups of *Rubus* and *Fragaria* and support the current phylogeny that places *Rubus* and *Fragaria* more closely to each other than either is to *Malus* or *Prunus*. The results of this study suggest that RLG1 and RLG6 have undergone rearrangements relative to the homologous *Fragaria* LG. The analysis of ancestral chromosome contribution suggests that of the four genera, *Prunus* shows the fewest rearrangements from the ancestral state. The insight into family-wide genome conservation gained by comparative mapping among these economically important genera provides useful information for Rosaceae crop geneticists and breeders. For example, gene homology location can be extrapolated from mapped orthologous markers and can be used to identify genes of interest in less well studied Rosaceae crops prior to the availability of genome sequences. The chromosome comparisons developed here will facilitate the assembly and annotation of future Rosaceae genome sequence projects.

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