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## The construction of a genetic linkage map of red raspberry (*Rubus idaeus* subsp. *idaeus*) based on AFLPs, genomic-SSR and EST-SSR markers

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**Abstract** Breeding in raspberry is time-consuming due to the highly heterozygous nature of this perennial fruit crop, coupled with relatively long periods of juvenility. The speed and precision of raspberry breeding can be improved by genetic linkage maps, thus facilitating the development of diagnostic markers for polygenic traits and the identification of genes controlling complex phenotypes. A genetic linkage map (789 cM) of the red raspberry *Rubus idaeus* has been constructed from a cross between two phenotypically different cultivars; the recent European cultivar Glen Moy and the older North American cultivar Latham. SSR markers were developed from both genomic and cDNA libraries from Glen Moy. These SSRs, together with AFLP markers, were utilised to create a linkage map. In order to test the utility of the genetic linkage map for QTL analysis, morphological data based on easily scoreable phenotypic traits were collected. The segregation of cane spininess, and the root sucker traits of density and spread from the mother plant, was quantified in two different environments. These traits were analysed for significant linkages to mapped markers using MapQTL and were found to be located on linkage group 2 for spines and group 8 for density and diameter. The availability of co-dominant markers allowed heterozygosities to be calculated for both cultivars.

### Introduction

*Rubus*, one of the most diverse genera in the plant kingdom, is subdivided into 12 subgenera (Jennings 1988), with ploidy levels ranging from diploid to 14-ploid (Nybom 1986). The domesticated subgenera contain the raspberries, blackberries, arctic fruits and flowering raspberries, all of which have been utilised in breeding programmes. The most important raspberries are the European red raspberry, *Rubus idaeus* L. subsp. *idaeus*, the North American red raspberry *R. idaeus* subsp. *s. trigosus* Michx and the black raspberry (*R. occidentalis* L.).

Roach (1985) and Jennings (1988) gave accounts of the early domestication of red raspberry (*R. idaeus*). Five parent cultivars dominate the ancestry of red raspberry; ‘Lloyd George’ and ‘Pynes Royal’ entirely derived from the European subspecies and ‘Preussen’, ‘Cuthbert’ and ‘Newburgh’ derived from both European and North American subspecies. Domestication has resulted in a reduction of both morphological and genetic diversity in red raspberry (Haskell 1960; Jennings 1988) with modern cultivars being genetically similar (Dale et al. 1993; Graham and McNicol 1995).

Breeding in raspberry, a highly heterozygous perennial fruit crop with relatively long periods of juvenility (Sanford 1983; Galletta 1983; Jennings 1988; McNicol and Graham 1992), is a long process. The speed and precision of breeding can be improved by the development of genetic linkage maps. Such genetic linkage maps can facilitate the development of diagnostic markers for polygenic traits and the identification of genes controlling complex phenotypes. To date, no genetic mapping work has been published on *Rubus*. Although considerable progress has been achieved in small (soft) fruit molecular biology, progress has not kept pace with developments in major crop plants (Hokanson 2001). Molecular marker applications have been reviewed in *Rubus* (Antonius-Klemola 1999) and in the small fruits (Hokanson 2001). Linkage maps have also been generated in other woody species (Ritter et al. 1990; Grattapaglia and Sederoff 1994;

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Bradshaw et al. 1994; Bradshaw and Stettler 1994). In the small (soft) fruit crops few maps exist, with those that do relying almost solely on randomly amplified polymorphic DNA (RAPD) markers (Hokanson 2001). In the diploid strawberry (*Fragaria vesca*) and diploid blueberry (*Vaccinium* spp.) 445 and 950 or 1288 cM long linkage maps based on RAPD markers have been constructed (Davis and Yu 1997; Rowland and Levi 1994; Qu and Hancock 1997).

*Rubus idaeus* has the potential to serve as a model species for the Rosaceae since it is diploid ( $2n=2x=14$ ) and has a very small genome (275 Mb). The availability of a detailed genetic linkage map, together with a deep coverage bacterial artificial chromosome library would be of great value in the identification of the genetic factors that underpin a wide range of commercial characteristics such as appearance, texture and sensory (taste and aroma) attributes of fruit, pest and disease resistance and a range of plant architectural traits. The establishment of gene-phenotype relationships will allow gene-based selection in breeding and the functional assignment of genes for fruit quality. The availability of a map would provide the basis to locate and hence manipulate quantitative traits in breeding programmes. The availability of informative mapped microsatellite markers, including functional EST-derived SSRs, will also allow selective genotyping of the pedigrees within raspberry breeding programmes. This would allow allele transmission between generations to be accurately monitored with transmission disequilibrium tests (TDT) being used to identify alleles contributing to commercially important attributes (Spielman et al. 1993). In this manuscript, we report the construction of a linkage map for *Rubus* based on linkage analysis, using a full-sib family approach for outbreeding species (Maliepaard et al. 1997). Both dominant (AFLPs) and co-dominant (SSR) markers have been developed, providing a long-term resource for breeding, map-based cloning and association mapping based on linkage disequilibrium. The utility of the map is demonstrated by mapping easily scoreable morphological traits of commercial interest.

## Materials and methods

### Mapping population and phenotypic analyses

A full-sib family from a cross between the European red raspberry cv. Glen Moy and the North American red raspberry cv. Latham, estimated to be around 60% similar (Graham and McNicol 1995) was used for mapping purposes. Latham was one of the first cultivars produced through controlled breeding in the 1930s, and is an exceedingly hardy, extremely spiny, brown-caned, small-fruited plant with resistance to raspberry root rot and a range of cane diseases. Glen Moy, released in 1981, in contrast, is large-fruited with good flavour and good sensory characteristics, susceptible to low temperature damage, spine-free and green-caned with little pest and disease resistance.

The entire segregating population of 300 individuals and both parents were cloned by the propagation of root material and planted at two field locations in randomised complete block trials with three replicates and two plant plots at both locations. A further replicate of the material was grown under protected cultivation in a screen tunnel.

### Inheritance of the “spiny phenotype”

Breeding for spinelessness is a major concern for breeders and there are several major genes that confer this trait (Jennings and Ingram 1983; Jennings 1988). Glen Moy, a spine-free genotype, is homozygous for gene *s* (Jennings 1988). Latham is very spiny, though the gene(s) underlying this have not been determined. Progeny from the mapping cross segregated for the degree of spines and were scored in both field locations and in a screen tunnel. Scoring was on a 1–5 scale, 1 being sparsely spiny (no individuals being spine-free) and 5 being densely spiny.

### Root sucker production

Large differences exist in the extent of root sucker production in cultivated raspberries. Control measures based on the chemical burning of early canes produced from suckers are required in commercial plantations to optimise fruit yield (Jennings, personal communication). The density and the spread of root suckers from the mother plants were each recorded on a 0–5 scale (0 being no production) under field conditions at both locations. For density scores, 0= no root suckers, 1=1–4 suckers, 2=5–8 suckers, 3=9–20, 4=21–40 and 5=>40 suckers. For root sucker spread, distance of root suckers from the mother plant was estimated, a score of 1 being suckers up to 10 cm from the mother plant and 5 being suckers at 1 m or greater from the mother plant

### Molecular marker development

#### Generation of AFLP markers

Total genomic DNA from the parents and 94 progeny was extracted using a 2% CTAB method (Graham et al. 2003). The AFLP template was prepared with either *Pst*I/ *Mse*I or *Eco*RI/ *Mse*I according to AFLP Analysis System II (Life Technologies) using 100 ng template DNA digested with *Eco*RI, *Pst*I and *Mse*I (Ellis et al. 1997). Pre-amplification reactions were performed with ‘core’ primers E00, 5’ GACTGCGTACCAATTC or P00 5’GACTGCGTACATCCAG and M00 5’GATGAGTCCTGAGTAA. Selective amplification was performed using primers with 2, 3 or 4 base extensions (*Pst*I- Preamp primer plus AC, AG, AT; *Eco*RI-Preamp primer plus AGC, AGG ; and *Mse*I-Preamp primer plus AAA, AGA, AGC, AGT, ATA, CAT, CGA, CGT, CTC, CTG, AAAA and ACGG) resulting in a total of 17 *Pst*I/ *Mse*I and 14 *Eco*RI/ *Mse*I primer

**Table 1** Polymorphic SSR primer pairs developed from genomic DNA from Glen Moy

Code	SSR	Left primer	Right primer	Allele sizes (bp) Latham	Allele sizes Glen Moy
Rubus1b	(ta)12-(ag)10	cctcttcaccgatttagacca	ttagcccccagtcctcaaaagt	212, 234	200, 229
Rubus2a	(gt)12-g-(gt)8	tgagggaagaagagcaaga	cacgtgtgaccaccaatgata	175, 180	180, 188
Rubus4a	(ct)14(ca)16	agcgaattgcatctctctctc	gcactgaaaaatcatgcatctg	143	139, 143
Rubus6a	(ct)16(ca)32	tgcatgtgactttgcatctct	gcactgaaaaatcatgcatctg	139	139, 150
Rubus12a	(ct)7(at)6(gt)10	attccccgcctcagaataat	aagggtttgtgacgggaacag	130, 150	141
Rubus16a	(at)8(gt)11	tgtgtacgtgttgggcttt	gggtgtttgccagtttcagt	145, 155	145, 155
Rubusr19a	(ta)5	gcagatcaatgaaagccatt	cggatcctccaacctcat	189, 199	201
Rubus20a	(a)14	tgacataatgcatgaaggga	cactatggttggccacagg	139	150, 154
Rubus22a	(at)16(gt)5	tgtggacgaccataactgc	tcggcattatacacacacaca	140, 150	150
Rubus24a	(at)5	acacacgcacgtacagcact	gcgcagtaagtggactttt	155, Null	166
Rubus25a	(gt)8	gccaaacacaccgttatcttg	cattaccacacgcttgatgc	144, 150	144
Rubus26a	(ct)11(ca)29	aacaccgcttctaaggtct	gatcctggaaagcgatgaaa	122, 150	150
Rubusr35a	(ct)8	ttggaagcacaagcgata	gcgacagccaaaacaaagt	210, 226	226
Rubusr43a	(ct)5	tgcttaaagttgctgctga	tcgaatgtaactcgcagtg	201, 209	201
Rubus45c	(t)10-(a)11-(ga)15	gaggggcaattaaagggtt	tgtgttaalttggttatcctgg	215, 245	228, Null
Rubusr47a	(ct)7-(ta)7	aagcaggacacctcagatgc	cagccaacctcatcagcta	203, 244	203
Rubus49a	(ta)7-(ga)7	cagccaacctcatcagcta	tgttttcaggaggcaggac	166, 182	166, 182
Rubusr56a	(tg)12(ag)11	tggagattccaataaacaataacc	tgtgtaaacctgggatgaa	212, 245	212
Rubus57a	(ag)11	atgtgtggggaagataacg	tgtcccaacatttcatacaaa	155	155, 185
Rubusr59b	(ct)9	ctcctcctcttctcctgca	aagtgtctgtatgtgtgc	279, 240	279
Rubusr76b	(ct)5-(ct)4	ctcaccgaaatgtcaacc	ggctaggccgaatgactaca	250, 340	250, 340
Rubus98d	(gaa)5-(ga)6	ggcttctcaatttctgtgtc	tgattgaaatctgctgggta	173, 178	178
Rubus102c	(c)9(a)10	cccctcccctctctgtatg	tcattgtgcaaacctgtacac	95, 123	123
Rubus105b	(ag)8	gaaaatgcaaggcgaattgt	tccatcaccaaccacccta	158, 190	158, 164
Rubus107a	(ag)8	gccagcaccacaaacctaca	ttcaccgtcaagaagaaagc	155, 160	155
Rubus110a	(tc)8	aaacaaggataaaagtgggaagg	tgtcagttggaggagaaca	180, 157	157
Rubus116a	(ct)12-(t)10	ccaacccaaaaacctcaac	gtgtggcatggcctttat	185, 190	193
Rubus117b	(cata)6-(ga)8	ccaactgaaacctcatgcac	acttggctctgtgtctgg	120, 155	132
Rubus118b	(ct)25	ccgcaaaacaaaaggcaag	ggattctgccaagtcgaa	104, 112	137
Rubus119a	(ga)8	gagcaaaacaaacacagatcaaa	ctcaaagtgtcagcagca	147, 150	147
Rubus123a	(ag)8	cagcagctagcattttactgga	gcactctccaccattcat	170, Null	140, 150
Rubus124a	(at)9	atgagcgcgaaatgtggat	gtggaagtgtgtcgtca	150, 162	150
Rubus126b	(ct)31(ca)22	cctgcattttctgtattttgg	tcagttttctccacgggta	150, 164	164, 201
Rubus137a	(tg)8-(ta)4	tgtgagcagagtgaaggagcta	agcattatctgcgcagttt	179, 187	179
Rubus145a	(gt)7	tgtcccagctttctgtttc	ggcatctgtcggtaaaaaat	131	139, 142
Rubus153a	(gt)11	cccagcttcagttggaaaga	agaggctcattgacctgaa	154, 156	156
Rubus160a	(ct)7	tccaactcggattctccatc	tatgtgagctgggcatggt	155, 160	155
Rubus163a	(ga)35	tgtgtcctctgcaaccatt	gcatagcccacaattagcaa	203	203, 207
Rubus166b	(tc)15	ccgcaagggtgtatctcaa	gcatgagggcgatataaagg	215, 221	219, 224
Rubus167a	(tc)9	aaccctaagccaaggacat	caccacctgatcagtcaga	166, 182	164, 180
Rubus194 h	(ga)12	tgtgtgttctctgcaacca	agcccttactttctgcaa	100, 111	111, 115
Rubus210a	(ct)25	tcctgatggtgtctggttg	ttcaggctttcagaacaa	101, 115	101, 115
Rubus223a	(at)4-(ta)8-(at)10	tctctgcatgttgagattctatt	ttaaggctcgtggataagg	138, 146	151
Rubus228a	(ga)41	tggacagctttgtgcagagt	gcttctgtatctccattgc	118, 128	150
Rubus233a	(ct)11	tgtctgtttgtattttgtgc	ggccaacaactctggataatca	190, 200	190
Rubus237b	(ttttc)3	catgctgcatgaccac	tgagccataaatttagaggatt	134	136, 147
Rubus243a	(ct)12	tgagcgagatgattggagtg	tatgtgtgcatatgcaagc	140	140, 174
Rubus251a	(ga)10	gcatcagccattgaatttcc	cccacctcattaccaactc	157, 183	157
Rubus252a	(ta)7-(ag)7	cattggctacaggcaactca	tggcacaagtgacagaag	128	128, 140
Rubus253a	(ct)34(at)11	acctccaatgccatagtgc	caagaatctgatctcgttagca	153, 165	153
Rubus256e	(ct)7(ct)8(at)10(ac)5	caacctgaaaacaaactcg	ctgagagcctgagaggtgt	172, 211	141
Rubus257a	(ct)4-(ct)6	ctcatcccaacaggtgtacg	gagactccatggcgagaag	181, 185	158, 185
Rubus259f	(ct)4-(ag)8	tggcacaagaagcctgtaac	tccatattcctcagcattc	244, 252	247

**Table 1** (continued)

Code	SSR	Left primer	Right primer	Allele sizes (bp) Latham	Allele sizes Glen Moy
Rubus260a	(ga)13	ttcgaatttcggatcaaac	gagagatctgacttgccaacg	165, 173	147
Rubus262b	(ag)15	tgcataagagcgatataaagg	tccgcaagggtgtatccta	217, 225	225
Rubus263f	(at)16-(ca)4	attccgccctgcataaatc	ggaaattggaaaccattgga	241, 253	253
Rubus264b	(ga)5-(gaaa)3(ga)7	tgcacagtttagggcaaaatc	atcaggctgcatttttacgc	175, 186	175, 186
Rubus268b	(ga)10	ccaagacaatgacctgagca	ggacagggtccacagagtg	177, 183	183, 204
Rubus270a	(ga)10	gcatcagccattgaatttc	cccactccattaccaactc	167, 185	158
Rubus275a	(ag)27	cacaaccagtcggagaat	catttcatcctcaaatgcaacc	130	114, 145
Rubus277a	(a)11(ag)8	gccccatcctgtacaagaa	ttgcaacaaaggtagtaatgg	234, 250	234
Rubus279a	(ga)21	tcgacatggctagttctacacag	ccccacttaaacattctca	110, 132	120
Rubus280a	(ag)13	ttcgaatttcggatcaaac	cgacaaaaaggaactcagc	183, 191	165
Rubus285a	(tc)9	tcgagaagctgctatgctg	ggatactcaatggctttctg	167, Null	138

combinations. One primer was end-labelled with gamma  $^{33}\text{P}$ . AFLP amplification products were denatured, resolved on 6% acrylamide gels run for approximately 3 h in Sequigen GT Nucleic Acid Electrophoresis Cells (Biorad).

#### Genomic SSR development

SSRs were identified as described by Graham et al. (2002). DNA was extracted from the red raspberry cultivar Glen Moy using a 2% CTAB method (Graham et al. 2003).

Ten micrograms of DNA was digested overnight with *Pst*I, size selected and fragments between 300 bp and 1.2 kb were ligated into dephosphorylated pUC18 and transformed into XLI-Blue supercompetent cells (Stratagene, Cambridge). Transformed colonies were picked into 384-well plates and colony filters constructed. A total of 12,288 colonies were screened with DIG-labelled AC<sub>(13)</sub> or AG<sub>(13)</sub> oligomers using a DIG nucleic acid detection kit (Roche) and 258 positive clones were sequenced on an ABI Prism 377 automated sequencer using the BigDye terminator cycle sequencing reaction ready kit. Primer pairs were designed to the flanking sequences of the repeat region using Primer 3 software (Rozen and Skaletsky 1998) and synthesised by Genosys. PCR reactions were done on 20 ng DNA from 94 progeny from the F<sub>1</sub> cross and the two parents in 25 µl reaction with 2 µM of each primer, 200 µM of each nucleotide, 1.5 mM of MgCl<sub>2</sub> and 0.5 U *Taq* DNA polymerase (Roche) per reaction, in a Gene Amp PCR System 9700 thermal cycler (Applied Biosystems) for 25 cycles with denaturation at 94 °C for 45 s, annealing at 59 °C for 45 s and extension at 72 °C for 1 min, with a final extension step of 5 min at 72 °C. For mapping, the 5' primers were fluorescently labelled with HEX, FAM or TET and PCR products were prepared according to Macaulay et al. (2001) for analysis on the ABI Prism 377. Allele sizes were determined using GENESCAN software programme (Applied Biosystems) and GeneScan-350 (Tamra), as an internal size standard.

#### Generation of EST-SSR markers

For SSR isolation from cDNA, mRNA was isolated from roots, leaves and fruit of the cultivar Glen Moy as described in Woodhead et al. (1997). cDNA was synthesised using a ZAP-cDNA Synthesis Kit and cloned using a Zap-cDNA GigapackIII and Gold Cloning Kit (Stratagene, Cambridge). After mass excision, colonies were picked into 384-well plates and 100 leaf, 100 root and 384 fruit colonies were sequenced on an ABI Prism 377 automated sequencer using the BigDye terminator cycle sequencing reaction ready kit. Twenty SSRs were identified and primer pairs were designed to the flanking sequences of the repeat region using Primer 3 software (Rozen and Skaletsky 1998), and synthesised by MWG Biotech (Munich). PCRs were carried out as for genomic SSRs except the left primer was end-labelled with gamma  $^{33}\text{P}$  by incubation for 1 h at 37 °C and 15 min at 75 °C with 0.1 U T4 polynucleotide kinase and 10× buffer. Amplification products were denatured then resolved on 6% acrylamide gels run for approximately 3 h in Sequigen GT nucleic acid electrophoresis cells (Biorad). Sequences were quality scored using Phred software (<http://www.phrap.com>) and then searched against the non-redundant nucleotide databases at NCBI (<http://www.ncbi.nlm.nih.gov>) using the BLAST algorithm (Altschul et al. 1990).

#### Linkage map construction and QTL analysis

Segregation analysis was carried out on the parents and 94 of the 300 progeny produced. Segregation data derived from polymorphic SSR and AFLPs in the Glen Moy × Latham cross were analysed using Joinmap v 2.0 for outbreeding populations (Stam and Van Ooijen 1995). The Kosambi function was used to convert recombination units into genetic distance. The mapping analysis was conducted using a LOD score of 6.0. MapQTL (v 3.0) (Van Ooijen and Maliepaard 1996) was used to run a Kruskal-Wallis analysis to detect markers with significant linkage to the phenotypic data collected for spines, root sucker density and root sucker spread. Interval mapping was then

**Table 2** Polymorphic SSR primer pairs developed from c-DNA

EST	SSR	Size	Left primer	Right primer	L × M	Homology
RubfruitE4	(ggttt)10	161	tcaaggtcctgctttaacc	cgcagagttggagacatgaa	abxaa	AP004942 Transcription factor
RubfruitE8	(ct)4(ta)4(ct)3	186	cataacctccagtgttcc	tgatgatgtcgcagcaggtt	abxaa	AF220491 o-Methyltransferase
RubfruitG7	(ct)22	159	ggcacgagtcaagctctct	tggttgggtttggaagca	abxaa	No homology
RubfruitC1	ctt-(cct)7	163	cacgagctcatcctctcc	atccaaagcttttgagattg	abxac	AY063105 Ribosomal protein 1
Rubleaf86	(gt)9	235	ggaactttggagccacctac	tcggcaggttattcattgt	abxaa	AJ131096 Heterotrimeric G protein
Rubleaf97	(ctt)-(cct)9	205	aacaaagctcctcgaccaga	tcgagatggctcagccaaca	abxaa	ATCYC2B Cyclin 2b protein
Rubleaf107	(ta)10	219	gcggcaagctcatatactcc	ggcaagcttgaatgaatcct	abxaa	No homology
Rubleaf102	(cat-)16	230	gcacgagcctcatctctc	gatgttcggcctttttaa	abxaa	No homology

conducted on the basis of the Kruskal-Wallis analysis after phase determination of markers using an interval of 1 cM and a maximum of ten neighbouring markers given the population type (Maliepaard and Van Ooijen 1994).

## Results

### Marker analysis

A total of 100 genomic SSR primer pairs were tested for polymorphism between the parents of the mapping population. Ten were monomorphic, 15 homozygous in both parents and nine primer pairs did not amplify products. A total of 66 polymorphic SSR markers were identified (Table 1). Fifteen EST-SSR primer pairs were assessed, eight of which proved polymorphic (Table 2). For the AFLP markers, 17 *Pst*I/ *Mse*I and 14 *Eco*RI/ *Mse*I primer pairs were tested producing 358 scoreable products with an average of 12 and nine dominant markers per primer combination, respectively.

### Map construction

Segregation data from 432 loci derived from the Genomic-SSRs (66), EST-SSRs (8) and AFLPs (358) were analysed using Joinmap (v 2.0). Initially, male and female maps were constructed. The Latham map was constructed from a subset of 299 markers containing all the SSRs and the informative AFLPs for that parent, and the Glen Moy map using 178 markers of which 58 were SSRs. For the construction of the individual parental maps, 22 AFLP markers of the type  $ab \times ab$  ( $aa \times b-$ ) were excluded from the analysis. Interestingly, the majority of the AFLPs

generated were informative in the Latham parent. The groupings generated from both types of SSR markers were the same on both maps and, therefore, a combined map was constructed.

Initially, a preliminary combined map was constructed on the complete data set and the map examined after the first, second and third round of mapping. Of the 432 markers analysed, 417 were placed on the linkage map at a LOD score of 6.0 after the third round of mapping. In this case a genetic linkage map covering 465.8 cM was generated (Table 3) with seven linkage groups. LOD scores beyond 7.0 resulted in fragmentation of the linkage groups. Of the 417 markers mapped, 12 departed from Mendelian expectation at a probability of 0.01. These were distributed equally across two of the seven groups.

This preliminary map was then refined by the removal of markers inserted in round 3 of the analysis. Removal of markers inserted in round 3, resulted in the map presented in Table 4 and Fig. 2 with nine groups being generated from a data subset of 273 markers. Two of the seven groups produced from the preliminary map have split resulting in the nine groups. The goodness-of-fit criteria used to generate the map in Table 4 are more rigid than for Table 3. The map for Table 3 is constructed such that all markers are fitted regardless of the change in goodness-of-fit.

### Phenotypic data analysis

#### *Spiny phenotype*

Phenotypes segregating from the cross ranged from the very spiny phenotype of Latham to a very sparsely spiny phenotype. There were no progeny with the spine-free

**Table 3** The preliminary raspberry genetic map based on 417 markers mapped after rounds 1, 2 and 3 of analysis using Joinmap

Linkage group	cM	AFLPs	SSRs	EST-SSRs	Mean marker distance (cM)
1	127.7	110	20	0	0.99
2	131.2	143	25	3	0.77
3	88.9	48	8	3	1.51
4	45.5	10	1	0	4.1
5	28.2	19	1	2	1.28
6	28.9	16	3	0	1.52
7	15.4	3	2	0	3.08

**Table 4** The refined raspberry genetic linkage map based on 273 markers mapped after rounds 1 and 2 of analysis using Joinmap

Linkage group	cM	AFLPs	SSRs	EST-SSRs	Mean marker distance (cM)
1	131.1	33	8	0	3.20
2	125.3	36	7	1	2.85
3	103.6	19	2	2	4.50
4	88.9	24	5	1	2.96
5	75.9	24	3	0	2.81
6	75.2	13	0	0	5.78
7	70.9	30	3	0	2.15
8	62.1	17	0	0	3.65
9	56.0	10	2	0	4.67

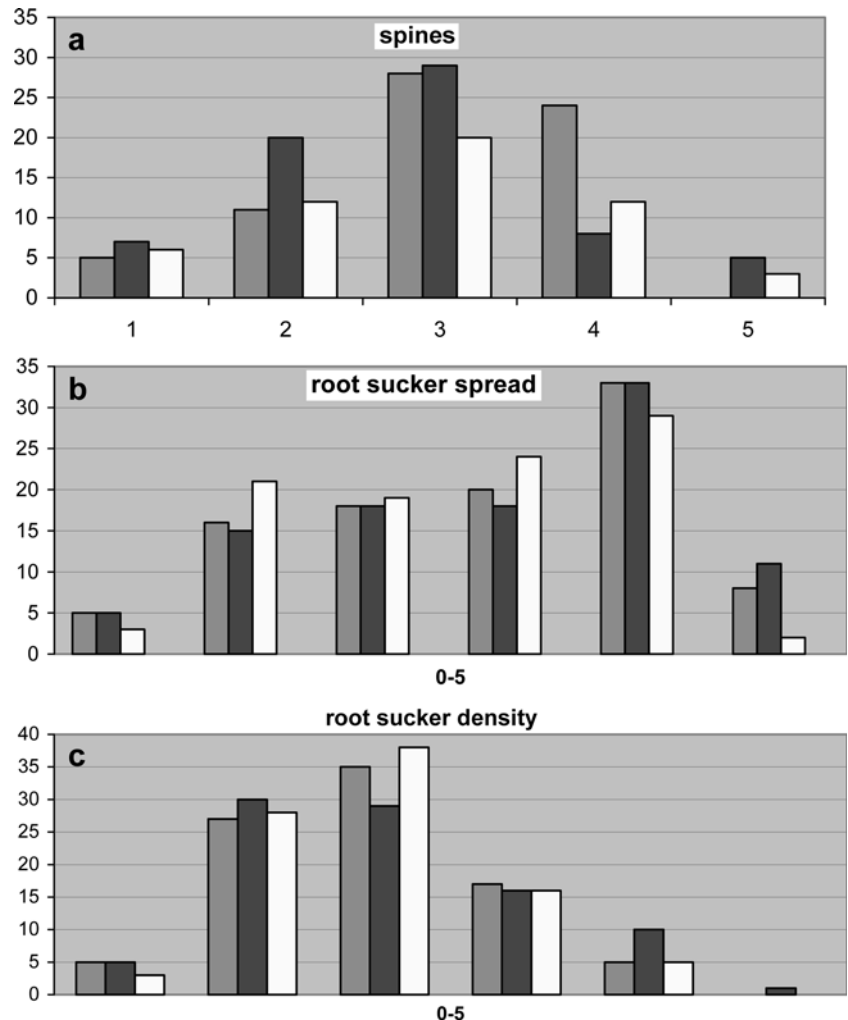
phenotype of Glen Moy, thus the extent of spines on the canes was recorded on a 1–5 scale (1 being sparse). The distribution of phenotypes at one location is given in Fig. 1a. Mean scores of spines were entered into MapQTL and 17 markers with significant linkages to the trait were identified at a probability of 0.0005–0.0001. Sixteen of the markers with significant associations to the trait locate to group 2 whereas the 17th was unmapped. Interval mapping was then carried out on group 2, where two regions were identified, the first between 46.5 and 56.1 cM, which accounted for 48% of the variation, and

the second between 108 and 120.9 cM, accounting for 50% of the variation (Fig. 2, linkage group 2).

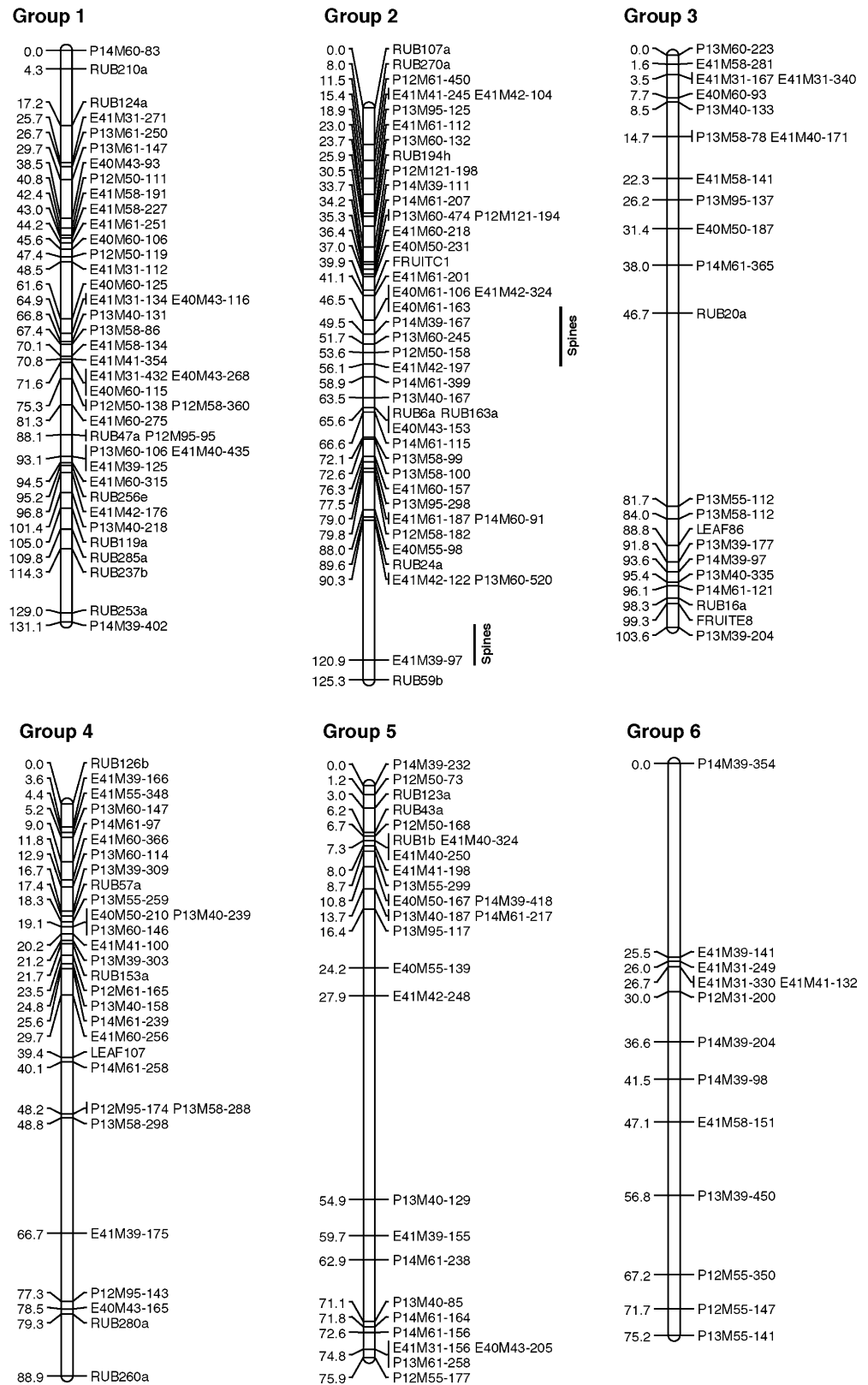
#### Root sucker phenotypes

The density and spread (from the mother plant) of root suckers were each scored on a 0–5 scale (Fig. 1b, c). MapQTL identified 14 markers with significant linkage to root sucker density and nine of these markers were also identified with significant linkage to root sucker spread.

**Fig. 1a–c** Distribution of field scores for the phenotypic data measures of spines (a) and root sucker measurements (b, c) of three replicates



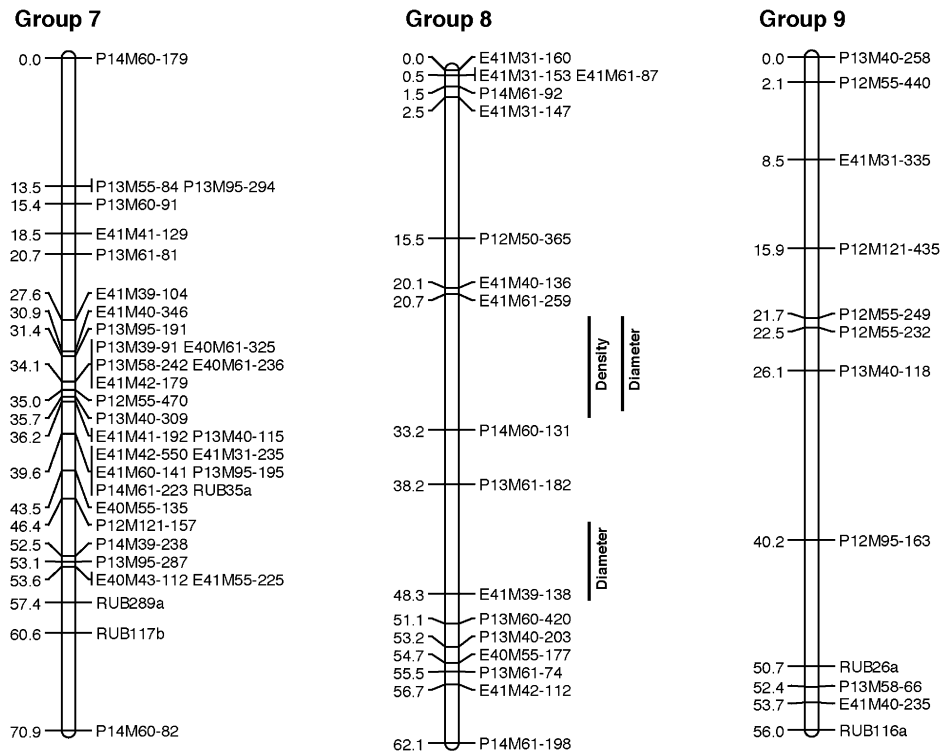
**Fig. 2** The genetic linkage map of red raspberry comprising nine linkage groups (1–9)



These markers were all located on group 8. Interval mapping located root sucker density between 22.7 and 30.7 cM accounting for 53% of the variation, and root sucker spread to two regions, the first between 44.2 and

50.3 cM accounting for 79% of the variation (Fig. 2, linkage group 8) and the second between 22.7 and 28.8 cM accounting for 33% of the variation.

Fig. 2 (continued)



### Estimate of heterozygosity

The development of co-dominant SSR and EST-SSR markers has allowed the evaluation of heterozygosity. On the basis of the loci identified being homozygous or heterozygous, mean heterozygosity of each parent was calculated to be 84% for Latham and 40% for Glen Moy.

### Discussion

This study is the first report of a genetic linkage map in raspberry. *R. idaeus* has a basic chromosome number of seven ( $2x=14=2n$ ) with chromosomes estimated to range from 1.3 to 2.8  $\mu\text{m}$  in length (1.3, 1.8, 2.0, 2.1, 2.2, 2.4 and 2.8  $\mu\text{m}$ ) (Poole et al. 1981). A preliminary genetic linkage map with seven groups was generated and then refined by the removal of markers causing a jump in the goodness-of-fit. This resulted in nine groups by splitting two of the groups generated for the preliminary map. The genetic linkage map produced is not at saturation. This is highlighted by 182 marker loci that could not confidently be placed on the map and the refined map having nine rather than seven groups. More markers are therefore required to generate seven groups and further marker development is underway to generate more EST-SSR markers.

Glen Moy has a spine-free phenotype (being homozygous for gene *s* (Jennings 1988)), whereas Latham is a densely spiny cultivar, the genetics of which has not been determined. The progeny generated from the cross were all spiny, though the extent of spines varied continuously from a very sparsely spiny cane to the densely spiny

phenotype of the Latham parent. From the phenotypic data we therefore propose that two or more genes are involved. This is supported by the mapping data where a number of markers were identified, linked to the spiny phenotypes. These markers are on linkage group 2, and there appears to be two linked regions within this group accounting for 98% of the variation. Interestingly we found that the very sparsely spiny phenotype (score 1) was associated with a bright brown cane, and we will aim to confirm this over each growing season for the next 3 years of the field trial. Breeding for spinelessness is a major concern of plant breeders, specifically when traits from old cultivars or species material are desired (Jennings, personal communication). There are several major genes conferring spinelessness; *s* (Lewis 1939), *Sf* (Rosati et al. 1986), *S<sub>TE</sub>* (Hall et al. 1986) and *Sf<sub>L</sub>* (McPheeters and Skirvin 1983; Rosati et al. 1988). A number of other genes are known to promote or suppress spines (Jennings 1988). Increased understanding of the genetic control of spiny phenotypes will be useful for future breeding.

Roots of red raspberry have adventitious buds, which develop on most roots. The number, density and distance from the mother plant of the root suckers varies between genotypes. Only a proportion of the buds normally develop into suckers. Knight and Keep (1960) have shown that the ability to produce suckers in red raspberry is determined by the recessive gene *sk<sub>1</sub>* or by the complementary genes *sk<sub>2</sub>* and *sk<sub>3</sub>*. Interestingly, and probably not surprisingly, the measurements of density and spread map to the same linkage group with an overlap in the location of the QTLs for the two traits.



## Estimate of heterozygosity

The genus *Rubus* is reported to contain a series of highly heterozygous species (Sanford 1983; Galletta 1983; Jennings 1988; McNicol and Graham 1992). The development of co-dominant SSR and EST-SSR markers has allowed the evaluation of heterozygosity in Latham and Glen Moy. It is not surprising that the value for Latham is significantly higher than for Glen Moy. Glen Moy is a more recently developed cultivar resulting from extensive breeding with genetically similar material, whereas Latham, an older cultivar, more closely resembles species material. Heterozygosity has been examined in a range of other fruit crops such as grapevine (Fatahi et al. 2003) strawberry (Sargent et al. 2003), sweet cherry (Struss et al. 2003) apple and pear (Hemmat et al. 2003).

The linkage map presented here will provide an excellent genetic framework for qualitative and quantitative trait analysis for the Rosaceae. A major future thrust will be to move from anonymous markers to functional polymorphisms based on EST-SSRs (Morgante et al. 2002) and SNPs (Rafalski 2002). Access to mapped markers will allow new approaches to breeding of complex traits that are difficult to manipulate in breeding programmes. In addition, the ability to retrospectively genotype pre-existing germplasm will allow the potential of association mapping in long-lived perennial crops to be evaluated as a tool for locating and eventually cloning genes determining traits of biological and economic importance.

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