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Over-seasons Analysis of Quantitative Trait Loci Affecting Phenolic Content and Antioxidant Capacity in Raspberry

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Supporting Information

ABSTRACT: This study examined the total phenol content (TPC) and total anthocyanin content (TAC) in ripe fruit of progeny of a mapping population generated from a cross between the European red raspberry cv. Glen Moy (*Rubus ideaus* var. *idaeus*) and the North American red raspberry cv. Latham (*Rubus ideaus* var. *strigosus*) over five seasons in two different growing environments. Measurements of antioxidant capacity (FRAP and TEAC) were also carried out. TPC was highly correlated with TEAC and FRAP across the entire data set. The subset of anthocyanin content was genotype-dependent but also correlated with TPC, although the proportion of anthocyanin compounds varied between progeny. Quantitative trait locus (QTL) analysis was carried out, and key markers were tested for consistency of effects over sites and years. Four regions, on linkage groups 2, 3, 5, and 6, were identified. These agree with QTLs from a previous study over a single season and indicate that QTL effects were robust over seasons.

KEYWORDS: *raspberry, progeny, polyphenols, anthocyanins, ellagitannins, antioxidants, inheritance, quantitative trait loci, environment*

INTRODUCTION

Berry fruit cultivation in the United Kingdom has relied for many years on new cultivars offering improvements in yield, cropping season, and resistance to damaging pests and diseases. Although these characters remain important to the success of newly released cultivars, there has been a growing demand from growers, processors, and consumers for improvements in fruit quality attributes, to the point where these traits are now equally important for cultivars and, indeed, may even affect decisions regarding commercial release.[1]

Fruit quality covers a range of traits, including physical characters such as berry size, berry color, berry conformation (drupelet structure and cohesion), firmness, and shelf life in the case of fresh fruit. Traits associated with chemical composition, such as color, sweetness, sourness, and flavor intensity, and the levels of nutritionally important compounds are becoming increasingly important.

A number of studies have been carried out in raspberry on quality aspects including a study of ripening,[2] color,[3] and anthocyanins.[4] These studies have examined environmental and seasonal effects on these traits as well as identified associated quantitative trait loci (QTLs), and, in some cases, candidate genes for their control have been hypothesized.

Berries are among the richest sources of polyphenols in commonly eaten fruits[5] and also provide a diverse range of polyphenols including flavonoids (such as anthocyanins, flavanols, and flavonols), condensed and hydrolyzable tannins, and phenolic acid derivatives.[6] In raspberries, the major polyphenols are anthocyanins and ellagitannins,[7−9] which make up >90% the total phenol cotent. The anthocyanins are responsible for their deep red coloration and are important targets for breeding efforts to improve and maintain consumer quality perception. Ellagitannins are important for the characteristic astringency and flavor of raspberries and must also be taken into account in breeding efforts.

Raspberry polyphenols have been implicated in a range of bioactivities relevant to human health.[10] Previous work has shown potent inhibition of cancer cell lines[8,11,12] and inhibition of digestive enzymes relevant to glycemic control,[13] lipid digestion, and obesity.[14] Indeed, in many cases, ellagitannins have been shown to be particularly potent.

This study examined the total phenol content (TPC) and total anthocyanin content (TAC) in ripe fruit of progeny of a mapping population generated from a cross between the European red raspberry cv. Glen Moy (*Rubus ideaus* var. *idaeus*) and the North American red raspberry cv. Latham (*Rubus ideaus* var. *strigosus*) over five seasons in two growing environments that differed in abiotic and biotic stresses. QTL analysis was carried out on the TPC and TAC to identify regions of the genome associated with these traits, and the consistency of key molecular markers for TPC and TAC over sites and years was examined.

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MATERIALS AND METHODS

The raspberry mapping population and genetic linkage maps have been described in detail previously.[2−4,15,16] It consists of a full sib family of 350 individuals generated from a cross between the European red raspberry cv. Glen Moy and the North American red raspberry cv. Latham.

Field Conditions. Two different trial sites were selected at the James Hutton Institute (JHI), Dundee, Scotland. The first (site H) was known to be contaminated by *Phytophthora rubi* (the causative agent of root rot), having previously been tested (JHI farm records), and the second (site B) was considered to be disease-free. Disease incidence and severity were further exacerbated at the contaminated site by spreading and rotavating contaminated topsoil from another site, irrigation on a daily basis (using a tape irrigation system from June until September), and planting in the absence of ridging and fungicide treatment. In contrast, the clean site (site B) was ridged as standard practice for growing raspberries to control *Phytophthora* and was treated with fungicides. Management at both sites was otherwise in line with current commercial practice. Both sites were planted in a randomized block design with three blocks per site. Further details of the trial sites and their management have been published[17] in a previous study of rot root resistance.

Fruit Sampling. Fruit was sampled from a single block at each site in 2003, 2004, 2005, and 2006. The fruit samples were frozen in bags until extraction. In 2007 and 2008, fruit was sampled from two blocks at the clean site (site B) only. In each year, fruit was sampled for the same 93 individuals. This subset of the cross has been referred to previously[2] and elsewhere as mapping population MP1, for which extensive molecular marker information is available.

Extraction Procedure. A representative subsample of fruit from each progeny was selected for extraction. The selected berries were cut in half, weighed, and then extracted with an equal volume to weight of acetonitrile containing 4% acetic acid. The samples were homogenized by hand using a glass tissue homogenizer with a PTFE pestle and then centrifuged at 13000 rpm for 5 min. The centrifugation was repeated and the supernatant taken as the extract. Subsamples and suitable dilutions were made for TPC and TAC measurements but also for FRAP and TEAC assays, which were carried out in batches. These extracts were stored at −80 °C.

Total Phenol and Total Anthocyanin Contents. TAC and TPC were estimated using the methods outlined previously.[18] In brief, TPC was measured using a modified Folin−Ciocalteu method with gallic acid as standard. TAC was estimated by a pH differential absorbance method. The absorbance value was related to anthocyanin content using a molar extinction coefficient calculated in-house for pure cyanidin-3-*O*-glucoside (purchased from ExtraSynthese, Genay, France). All analyses were carried out in triplicate.

Assessment of Antioxidant Capacity (TEAC and FRAP). Analyses were performed as described before.[18] For the TEAC assay, samples were mixed with buffer (25 mM phosphate, pH 7.4, 488.6 μL), metmyoglobin (70 mM stock in buffer, 36 μL), and 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS, 500 mM stock in buffer, 300 μL). Absorbance (734 nm) of the developing $ABTS^{\bullet+}$ chromophore was recorded 7.5 min after initiation by addition of hydrogen peroxide solution (450 μM stock in water, 167 μL). In controls, distilled water replaced the hydrogen peroxide. All analyses were carried out in triplicate.

A manual FRAP assay based on the method described previously[18] was used. FRAP reagent was freshly prepared (1 mM 2,4,6-tripyridyl-2-triazine (TPTZ) and 2 mM ferric chloride in 0.25 M sodium acetate, pH 3.6). A 100 μL aliquot of raspberry extract (at 1% v/v in distilled water) was added to 900 μL of FRAP reagent and mixed. After standing at ambient temperature (∼20 °C) for 4 min, absorbance at 593 nm was determined against a water blank. Calibration was against a standard curve (50 ± 1000 μM ferrous ion) produced by the addition of freshly prepared ammonium ferrous sulfate. FRAP values obtained are presented as micromolar ferrous ion equivalents (ferric reducing power) of the extracts, from three determinations.

Statistical Analysis. The TPC, TAC, FRAP, and TEAC data were analyzed using a mixed model fitted by residual maximum likelihood [REML][19] to estimate site and year means. The 93 individual genotypes were initially fitted as a random effect, as was the interaction between genotype and environment (i.e., year by site combination). A common residual variance across the environments was compared with separate residual variances to see whether there were significant differences among the environments. The broad-sense heritability for each trait was estimated as

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GL}^2 + \sigma_E^2}$$

where σ_G^2 is the variance component for genotypes, σ_{GL}^2 is the variance component for the genotype by environment interaction, and σ_E^2 is the overall residual mean square. The analysis was repeated with genotype and environment as fixed effects to estimate genotype means across the environments for each trait for QTL mapping. Each trait was mapped on the linkage map using Kruskal−Wallis analysis, as implemented in the MapQTL 5 software.[20] The most significant markers were included as fixed effects in a mixed model analysis to test for significant marker main effects and interactions with site and year.

All statistical analyses apart from the Kruskal−Wallis mapping were carried out using the statistical program Genstat 12 for Windows.[21] The linkage maps were drawn using MapChart 2.2.[22]

RESULTS AND DISCUSSION

The Pearson correlations between TPC, TAC, and the measurements of antioxidant capacity (FRAP and TEAC) over all seasons and fields are shown in Table 1. All of the correlations are positive and highly significant ($p < 0.001$). TPC and the two measures of antioxidant capacity (FRAP and TEAC) were more highly correlated with each other than with TAC. Table 2 shows the correlation between TPC and TAC at every site and year. TAC and TPC were significantly correlated at every site and year. For field B in 2005 and 2006, the correlation was lowest, but still significant with $p < 0.05$. For the other environments the correlation was significant with $p < 0.001$.

Table 1. Pearson Correlations (R) between Total Phenol Content (TPC), Total Anthocyanin Content (TAC), and Antioxidant Capacity Measurements (FRAP and TEAC) over All Seasons and Field Environments

TPC[a]			
FRAP[b]	0.81		
TEAC[b]	0.87	0.84	
TAC[b]	0.43	0.46	0.41
	TPC	FRAP	TEAC

[a]Expressed as mg/100 g FW fruit. [b]Expressed as mmol/g FW.

The two measurement of antioxidant capacity (TEAC and FRAP) correlated well with TPC. This has been noted before within varieties of cultivated berries and wild species of berries.[18] It is well accepted that the Folin assay for TPC and the antioxidant measurements (TEAC and FRAP) effectively measure different aspects of antioxidant capacity[23] and therefore are often well correlated within berry types.[24,25] TAC, which is a subset of total phenol content, also correlated with TPC and the measures of antioxidant capacity. This has been noted previously. For example, TPC was closely correlated with FRAP ($r = 0.93$) in progeny of factorial mating design experiment encompassing 411 raspberry genotypes,[26] but TAC was less well correlated with FRAP ($r = 0.53$) [27]

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Table 2. Mean ± Standard Deviation for Total Phenol Content (TPC), Total Anthocyanin Content (TAC), and Antioxidant Capacity Measurements (FRAP, TEAC) for All Environments and Pearson Correlation (R) between TPC and TAC

year	site[a]	TPC[b]	TAC[b]	FRAP[c]	TEAC[c]	R[d]
2003	B	129.4 ± 49.8	63.4 ± 35.9	15765.9 ± 4851.6	15.1 ± 4.0	0.44***
	H	131.6 ± 43.1	62.2 ± 33.1	15839.2 ± 5176.8	15.2 ± 4.3	0.40***
2004	B	170.9 ± 42.8	81.7 ± 30.2	19698.9 ± 5584.6	20.1 ± 4.6	0.38***
	H	184.6 ± 47.0	88.4 ± 33.9	20922.1 ± 6327.7	20.7 ± 4.6	0.58***
2005	B	143.1 ± 46.7	68.5 ± 22.1	18309.1 ± 6124.3	19.3 ± 5.0	0.24*
	H	171.8 ± 61.1	79.7 ± 30.1	22199.8 ± 7531.2	21.3 ± 5.9	0.47***
2006	B	175.0 ± 50.2	68.9 ± 22.0	19998.7 ± 6669.0	21.5 ± 5.2	0.22*
	H	149.7 ± 37.6	64.1 ± 23.5	15126.0 ± 4782.5	18.1 ± 4.3	0.38***
2007	B	150.3 ± 36.9	61.1 ± 24.7	14145.3 ± 5017.6	16.6 ± 4.2	0.31***
2008	B	183.1 ± 59.5	58.3 ± 27.2	21531.9 ± 7855.3	21.4 ± 6.2	0.41***

[a]Field sites B and H are discussed in the text. [b]Expressed as mg/100 g FW fruit. [c]Expressed as mmol/g FW fruit. [d]*, $p < 0.05$; ***, $p < 0.001$.

The correlation of TEAC and FRAP with TPC has been noted before within varieties of cultivated berries and wild species of berries.[18] It is well accepted that the Folin assay for TPC and the antioxidant measurements (TEAC and FRAP) effectively measure different aspects of antioxidant capacity[23] and therefore are often well correlated within berry types.[24,25] The correlations of these with TAC, which is a subset of total phenol content, have also been noted previously. For example, TPC was closely correlated with FRAP ($r = 0.93$) in the progeny of a factorial mating design experiment encompassing 411 raspberry genotypes,[26] but TAC was less well correlated with FRAP ($r = 0.53$).[27]

Despite the fact that anthocyanins are a subset of the polyphenolic pool, there is substantial plasticity in TAC compared to TPC in raspberry, which suggests that anthocyanin levels are not governed by the size of the total polyphenol pool. This plasticity has been highlighted in previous work on this raspberry progeny set[3,4] grown under field and controlled conditions. However, this study provides evidence that the plasticity is robust across multiple seasons and in two field environments. As anthocyanins are end-points of a branch of the general phenolic biosynthetic pathway (Figure 2), they are likely to be subject to different control mechanisms. Abiotic influences, such as light and temperature, have long been known to influence anthocyanin biosynthesis and accumulation (see, e.g., ref 28). Moreover, recent work has illustrated that altering postflowering temperature can influence anthocyanin content and composition and the amounts of ellagitannin components in raspberry.[29]

Table 2 shows the mean and standard deviations for each trait at each location. In the overenvironments mixed model for each trait, the deviance was reduced significantly ($p < 0.001$) when separate residual variances were fitted, rather than a common variance. This showed that the environmental variability differed between sites and years. The measurements of TPC, FRAP, and TEAC had the highest variability for 2005 site H and 2008 site B, whereas for TAC the most variable environment was 2003 site B. There was no significant change in the deviance for any of the traits when the genotype by environment interaction was dropped from the model, showing that this interaction is not significant, but for each trait the variance component for genotype was significant ($p < 0.001$). The traits showed moderate broad-sense heritability: 31.1% for TPC, 30.3% for FRAP, 35.3% for TEAC, and 35.7% for TAC. Although there were significant differences in the means of each trait among the environments ($p < 0.001$), there was no consistent difference between the H and B sites.

The differences in distribution can be illustrated by box plots (Supporting Information, Figure S1). Some genotypes have consistently high values: numbers 11, 184, 160, 127, and 19 occur as outliers in more than one environment for each of the TAC, FRAP, and TEAC measurements. There are no consistent outliers for TAC.

QTL mapping using Kruskal–Wallis analysis identified significant regions on four linkage groups in total (Figure 1). Markers on linkage group (LG) 2 were significant for TAC content only, whereas markers on LG 3 and LG 5 were significant for TPC, FRAP, and TEAC but not specifically for the anthocyanins. Markers on LG 6 were significant for all four traits.

The results for QTL mapping of TAC and TPC agree with previous work[3,30] that used a larger selection of the same mapping population in field and polytunnel sites but in only one growing season (2008). They identified QTLs for TAC near the QTL on LG 2 found in this study and at the same marker on LG 6. They also found QTLs for TAC in the regions of LG 3 identified in this study and QTLs for total phenol content on LG 3 and LG 5. They also identified QTLs for color that overlapped the QTLs for TPC noted on LG 6 in this study. On the other hand, the previous work found a QTL for TPC on LG 1 in polytunnel-grown progeny,[30] which was not detected here. In addition, the midpoint for the QTL for TAC content in LG 2 was slightly different in the previous study,[30] with P13M40-85 as the most significant marker.

The most significant marker for TAC on LG 2 was P13M95-298R, at 109cM, close to the marker bes_Ri29G13R at 100cM, which was reported[3] to be associated with total anthocyanin content. P13M95-298R is heterozygous (genotype ab) for the Latham parent and homozygous for the Glen Moy parent (genotype aa) and therefore segregates in an approximate 1:1 ratio of aa:ab genotypes in the offspring. The consistency of its relationship to TAC over years and sites was investigated by modeling TAC as a function of the P13M95-298R genotype, site, year, and the interactions between these. The effect of

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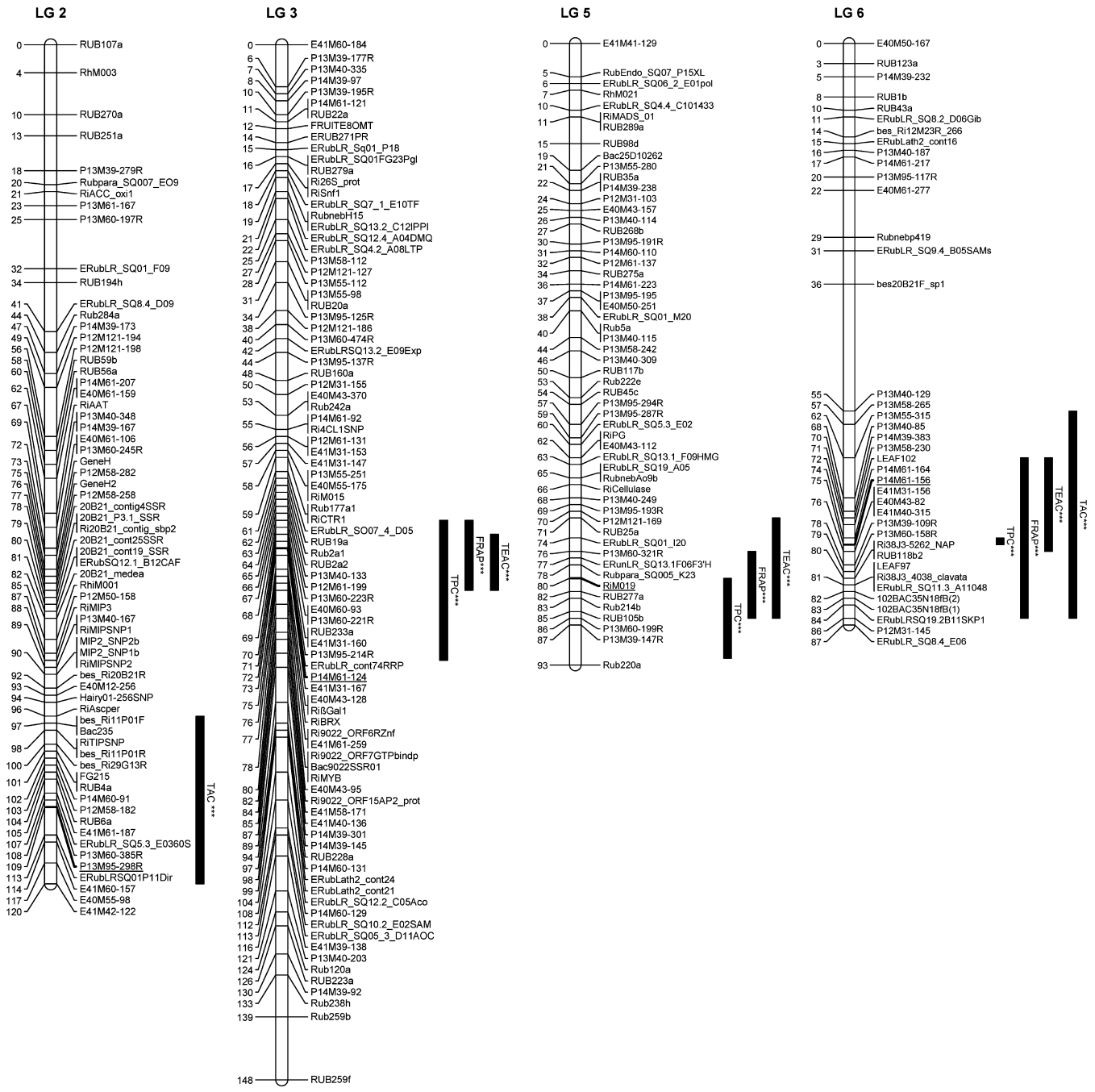

Figure 1. Maps of *Rubus* linkage groups 2, 3, 5, and 6. The map shows the regions where the significance of the Kruskal–Wallis test for associations with the traits is <0.001. The markers used in the mixed model are underlined.

P13M95-298R was significant with $p < 0.001$, but there was also a significant interaction with the site ($p = 0.002$). Offspring with genotype ab at this marker had significantly higher TAC than those with genotype aa, with a larger difference at the infected site H (Table 3). The most significant association with TPC, FRAP, and TEAC on LG 3 was with marker P14M61-124, at 72cM, which is also heterozygous (ab) for Latham and homozygous for Glen Moy. When this was included in a mixed model for TPC, its effect was significant with $p < 0.001$. There was also some evidence of an interaction between the marker, site, and year ($p = 0.033$). Offspring with genotype ab had significantly higher TPC than those with genotype aa, except for site H in 2003, where the differences were not significant. The mean difference, excluding site H in 2003, was 27.4 mg/100 g, with sed = 9.17. FRAP and TEAC showed similar relationships with this marker. The most significant association with TPC, FRAP, and TEAC on LG 5 was with marker RiM019 at 80cM, which is heterozygous for both parents with four different alleles (abxcd) and, therefore, four genotype classes for the offspring ac:ad:bc:bd occurring in an expected 1:1:1:1 ratio.. When this marker was included in a mixed model for TPC, its effect was significant with $p < 0.001$, but no interactions of this marker with year or site were significant. The genotype means were ac, 168.9; ad, 174.7; bc, 138.1; and bd, 156.5, with an average sed = 8.17. FRAP and TEAC showed similar relationships with this marker. For LG 6, marker

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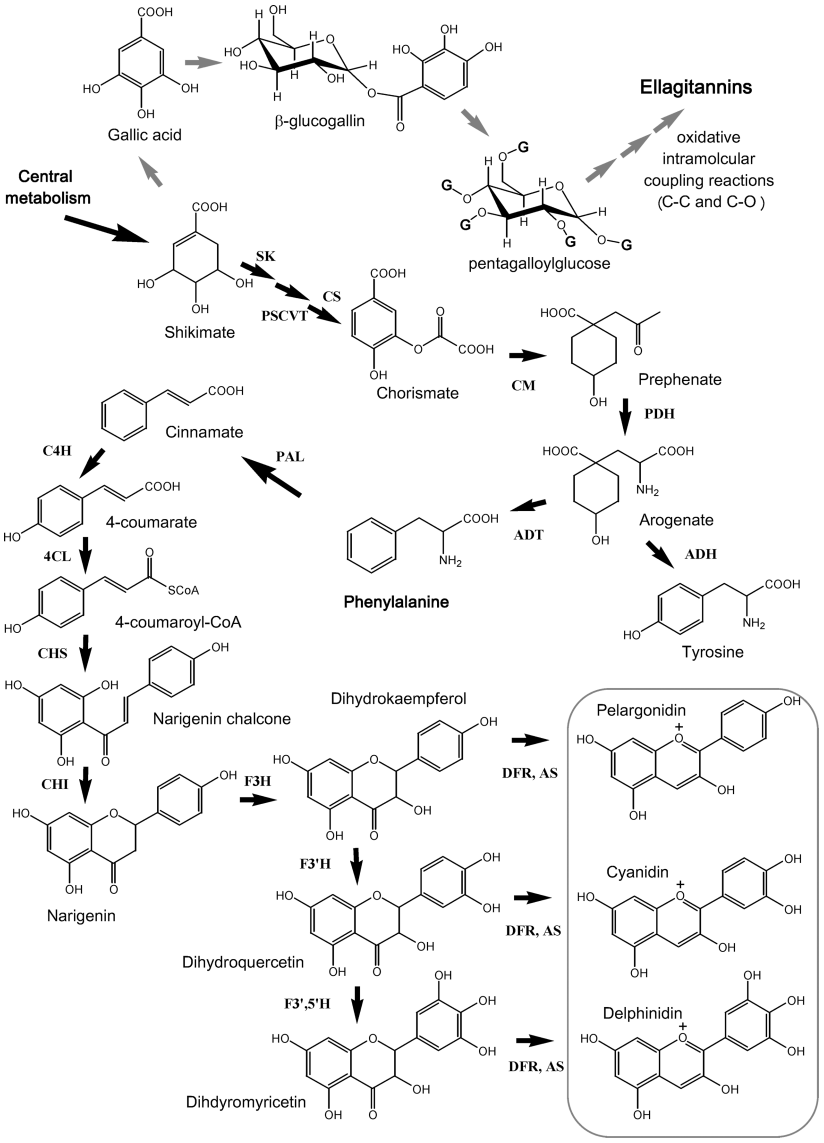

Figure 2. Overview of biosynthetic pathways for phenolic components in raspberry. Black arrows represent known enzymatic steps. Gray arrows represent postulated enzymatic steps. The anthocyanidins are shown in a box. Enzyme acronyms: ADH, arogenate dehydrogenase; ADT, arogenate dehydratase; AS, anthocyanin synthase; CHI, chalcone isomerase; CHS, chalcone synthase; C4H, cinnamate-4-hydroxylase; 4CL, 4-coumarate ligase; CM, chorismate mutase; CS, chorismate synthase; DFR, dihydroflavonol reductase; F3H, flavonone-3-hydroxylase; F3′H, flavonoid-3′-hydroxylase; F3′5′H, flavonoid-3′,5′-hydroxylase; PAL, phenylalanine ammonia-lyase; PDH, prephenate dehydratase; PSCVT, 3-phosphoshikimate 1-carboxyvinyl transferase; SK, shikimate kinase.

P14M61-156, at 75cM, which is also heterozygous (ab) for Latham and homozygous for Glen Moy, showed the strongest association with TPC, FRAP, and TEAC and was close to the strongest association with TAC. When this was included in a mixed model for TPC, its effect was significant with $p < 0.001$, and no interactions of this marker with year or site were significant. The genotype means were 145.9 for aa and 169.6 for ab, with average sed = 5.58. FRAP and TEAC showed similar relationships with this marker. For TAC, its effect was significant with $p < 0.001$, but there was also a significant association of this marker with the environment ($p = 0.004$). Individuals with genotype ab had significantly higher TAC than those with genotype aa in most environments, but the genotypes were not significantly different at site B in 2003 or at site H in 2005 or 2006 (Table 3).

The QTLs found here on LG 3 and LG 6 are in regions where QTLs for many traits have been detected, including traits for general vigor, ripening, and root rot resistance.[3,16] The detection of a QTL affecting TAC but not TPC on LG 2 agrees with the previous findings,[3] as does the detection of a QTL affecting TPC but not TAC content on LG 5.

The polyphenolic composition of raspberry is dominated by anthocyanin and ellagitannin components,[7−9] and therefore TPC minus TAC could be construed as a rough assessment of ellagitannin content The lower correlation between TAC and FRAP/TEAC than for TPC with these antioxidant measurements confirms previous work that strongly suggested that ellagitannins were the greatest contributors to antioxidant capacity in raspberry.[7,8,31,32] Indeed, ellagitannins have been implicated in many of the putative biological activities of raspberries.[7,8,12,14] Therefore, finding QTLs for TPC that are not shared by TAC may help to identify markers for ellagitannin accumulation and biosynthesis. This may be particularly useful as our understanding of ellagitannin

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Table 3. Mean Total Anthocyanin Content (TAC)[a] for the aa and ab Genotypes of Marker P13M95-298R (from Linkage Group 2) at Each Site and of Marker P14M61-156 (from Linkage Group 6) at Each Site and Year

(A) P13M95-298R Genotype				
site		aa	ab	sed[b]
B		59.59	73.94	3.446
H		61.55	84.77	3.917
(B) P14M61-156 Genotype				
year	site	aa	ab	sed[c]
2003	B	61.15	66.12	7.368
	H	53.20	70.74	7.369
2004	B	70.01	93.07	5.692
	H	76.69	99.31	5.867
2005	B	58.77	78.47	4.447
	H	76.44	83.61	5.870
2006	B	62.14	75.29	4.606
	H	66.36	63.88	4.989
2007	B	53.01	69.17	4.246
2008	B	49.77	65.46	5.899

[a]TAC is expressed as mg/100 g FW fruit. [b]The seds (standard error of difference) in the table are for comparison of the genotypes within each site. The average sed across all pairwise comparisons was 3.306. [c]The seds in the table are for comparison of the genotypes within each site and year. The average sed across all pairwise comparisons was 5.407.

biosynthesis is not well-defined[33] and is well behind that of anthocyanin biosynthesis (see, e.g., ref 34). Ellagitannins also contribute to sensorial quality through astringency and, along with acid/sugar balance, are key to the complex sensory nature of raspberries.[35] From what is known about the biosynthesis of ellagitannins, they originate from gallic acid, which is itself formed from the central metabolite, shikimate. Therefore, regulation of ellagitannin content must operate at a different level, "higher" up the biosynthetic pathway than the biosynthesis of anthocyanins, which effectively represents a metabolic end-point (Figure 2). Ellagitannins are generally synthesized earlier in fruit development than the anthocyanins,[36] which are obviously associated with ripening, and therefore must also come under different temporal control regimes.

In general, the QTL effects are quite consistent over years and sites: interactions are either nonsignificant or only weakly significant. Some differences were less significant in the 2003 sampling (the first fruiting year) than in later samples, which may reflect differences in plant maturity and fruit set. There is some evidence that anthocyanin QTLs may have different sized effects at clean and root rot sites, but this needs to be investigated further on a larger population before firm conclusions about this can be drawn. Therefore, we conclude that the molecular markers identified here are good candidates for use in marker-assisted selection.

■ ASSOCIATED CONTENT

Supporting Information

Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Graham, J.; Jennings, S. N. Raspberry breeding. In *Breeding Plantation Tree Crops: Tropical Species*; Jain, S. M., Priyadarshan, M., Eds.; IBH and Science Publication: Oxford, U.K., 2009; pp 233−248.

(2) Graham, J.; Hackett, C. A.; Smith, K.; Woodhead, M.; Hein, I.; McCallum, S. Mapping QTLs for developmental traits in raspberry from bud break to ripe fruit. *Theor. Appl. Genet.* **2009**, *118*, 1143−1155.

(3) McCallum, S.; Woodhead, M.; Hackett, C. A.; Kassim, A.; Paterson, A.; Graham, J. Genetic and environmental effects influencing fruit color and QTL analysis in raspberry. *Theor. Appl. Genet.* **2010**, *121*, 611−627.

(4) Kassim, A.; Poette, J.; Paterson, A.; Zait, D.; McCallum, S.; Woodhead, M.; Smith, K.; Hackett, C.; Graham, J. Environmental and seasonal influences on red raspberry anthocyanin antioxidant contents and identification of QTL. *Mol. Nutr. Food Res.* **2009**, *53*, 625−634.

(5) Kähkönen, M. P.; Hopia, A. I.; Heinonen, M. Berry phenolics and their antioxidant activity. *J. Agric. Food Chem.* **2001**, *49*, 4076−4082.

(6) Macheix, J.-J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC Press: Boca Raton, FL, 1990.

(7) Mullen, W.; McGinn, J.; Lean, M. J.; MacLean, M. R.; Gardiner, P.; Duthie, G. G.; Yokota, T.; Crozier, A. Ellagitannins, flavanoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vaso-relaxation properties. *J. Agric. Food Chem.* **2002**, *50*, 5191−5196.

(8) Ross, H. A.; McDougall, G. J.; Stewart, D. Antiproliferative activity is pedominantly associated with ellagitannins in raspberry extracts. *Phytochemistry* **2007**, *68*, 218−228.

(9) McDougall, G. J.; Martinussen, I.; Stewart, D. Towards fruitful metabolomics: high throughput analyses of polyphenol composition in berries using direct infusion mass spectrometry. *J. Chromatog., B* **2008**, *871*, 362−369.

(10) Seeram, N. P. Berry fruits: compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. *J. Agric. Food Chem.* **2008**, *56*, 627−629.

(11) McDougall, G. J.; Ross, H. A.; Ikeji, M.; Stewart, D. Berry extracts exert different antiproliferative effects against cervical and colon cancer cells grown in vitro. *J. Agric. Food Chem.* **2008**, *56*, 3016−3023.

(12) Coates, E. M.; Popa, G.; Gill, C. I. R.; McCann, M. J.; McDougall, G. J.; Stewart, D.; Rowland, I. R. Colon-available raspberry polyphenols exhibit anti-cancer effects on *in vitro* models of colon cancer. *J. Carcinogenesis* **2007**, *6*, 4−11.

(13) Grussu, D.; Stewart, D.; McDougall, G. J. Berry polyphenols inhibit α-amylase in vitro: identifying active components in rowanberry and raspberry. *J. Agric. Food Chem.* **2011**, *59*, 2324−2331.

(14) McDougall, G. J.; Kulkarni, N.; Stewart, D. Berry polyphenols inhibit pancreatic lipase activity *in vitro*. *Food Chem.* **2009**, *115*, 193−199.

(15) Graham, J.; Smith, K.; MacKenzie, K.; Jorgenson, L.; Hackett, C. A.; Powell, W. The construction of a genetic linkage map of red raspberry (*Rubus idaeus* subsp. *idaeus*) based on AFLPs, genomic-SSR and EST-SSR markers. *Theor. Appl. Genet.* **2004**, *109*, 740−749.

(16) Graham, J.; Smith, K.; Tierney, I.; MacKenzie, K.; Hackett, C. A. Mapping gene *H* controlling cane pubescence in raspberry and its association with resistance to cane botrytis and spur blight, rust and cane spot. *Theor. Appl. Genet.* **2006**, *112*, 818−831.

(17) Graham, J.; Hackett, C. A.; Smith, K.; Woodhead, M.; MacKenzie, K.; Tierney, I.; Cooke, D.; Bayer, M. Towards an understanding of the nature of resistance to *Phytophthora* root rot in red raspberry: is it mainly root vigour? *Theor. Appl. Genet.* **2011**, *123*, 585−601.
(18) Deighton, N.; Brennan, R.; Finn, C.; Davies, H. V. Antioxidant properties of domesticated and wild *Rubus* species. *J. Sci. Food Agric.* **2000**, *80*, 1307−1313.
(19) Patterson, H. D.; Thompson, R. Recovery of inter-block information when block sizes are unequal. *Biometrika* **1971**, *58*, 545−554.
(20) Van Ooijen, J. W. MapQTL® 5, software for the mapping of quantitative trait loci in experimental populations. Kyazma BV, Wageningen, The Netherlands, 2004.
(21) Genstat for Windows Release 12.2. VSN International Ltd., Hemel Hempstead, Hertfordshire, U.K., 2009
(22) Voorrips, R. E. MapChart: Software for the graphical presentation of linkage maps and QTLs. *J. Heredity* **2002**, *93*, 77−78.
(23) Huang, D.; Ou, B.; Prior, R. L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* **2005**, *53*, 1841−1856.
(24) Wang, S. Y.; Lin, H.-S. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.* **2000**, *48*, 140−146.
(25) Sariburun, E.; Şahin, S.; Demir, C.; Türkben, C.; Uylaşer, V. Phenolic content and antioxidant activity of raspberry and blackberry cultivars. *J. Food Sci.* **2010**, *4*, C328−C335.
(26) Connor, A.-M.; Stephens, M. J.; Hall, H. K.; Alspach, P. A. Variation and heritabilities of antioxidant activity and total phenolic content estimated from a red raspberry factorial experiment. *J. Am. Hortic. Sci.* **2005**, *130*, 403−411.
(27) Connor, A.-M.; McGhie, T. K.; Stephens, M. J.; Hall, H. K.; Alspach, P. A. Variation and heritability estimates of anthocyanins and their relationship to antioxidant activity in a red raspberry factorial mating design. *J. Am. Hortic. Sci.* **2005**, *130*, 534−542.
(28) Jaakola, L.; Hohtola, A. Effect of latitude on flavonoid biosynthesis in plants. *Plant Cell Environ.* **2010**, *33*, 1239−1247.
(29) Remberg, S. V.; Sønsteby, A.; Aaby, K.; Heide, O. M. Influence of postflowering temperature on fruit size and chemical composition of Glen Ample raspberry (*Rubus idaeus* L.). *J. Agric. Food Chem.* **2010**, *58*, 9120−9128.
(30) McCallum, S. Marker assisted breeding for sensory characteristics in red raspberry (*Rubus idaeus*). Ph.D. thesis, University of Strathclyde, 2010.
(31) Beekwilder, J.; Jonker, H.; Meesters, P.; Hall, R. D.; van der Meer, I. M.; de Vos, C. H. R. Antioxidants in raspberry: on-line analysis links antioxidant activity to a diversity of individual metabolites. *J. Agric. Food Chem.* **2005**, *53*, 3313−3320.
(32) Borges, G.; Degeneve, A.; Mullen, W.; Crozier, A. Identification of flavonoid and phenolic antioxidants in black currants, blueberries, raspberries, red currants, and cranberries. *J. Agric. Food Chem.* **2010**, *58*, 3901−3909.
(33) Gross, G. G. Biosynthesis of ellagitannins: old ideas and new solutions. In *Chemistry and Biology of Ellagitannins. An Underestimated Class of Plant Poylphenols*; Quideau, S., Ed.; World Scientific Publishers: London, U.K., 2009; pp 94−118.
(34) Lin-Wang, K.; Bolitho, K.; Grafton, K.; Kortstee, A.; Karunairetnam, S.; McGhie, T. K.; Espley, R. V.; Hellens, R. P.; Allan, A. C. An R2R3MYB transcription factor associated with regulation of the anthocyanin biosynthetic pathway in Rosaceae. *BMC Plant Biol.* **2010**, *21*, 10−15.
(35) Bakkalbaşi, E.; Menteş, O.; Artik, N. Food ellagitannins − occurrence, effects of processing and storage. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 283−298.
(36) Fait, A.; Hanhineva, K.; Beleggia, R.; Dai, N.; Rogachev, I.; Nikiforova, V. J.; Fernie, A. R.; Aharoni, A. Reconfiguration of the achene and receptacle metabolic networks during strawberry fruit development. *Plant Physiol.* **2008**, *148*, 730−750.