Supplementary figures

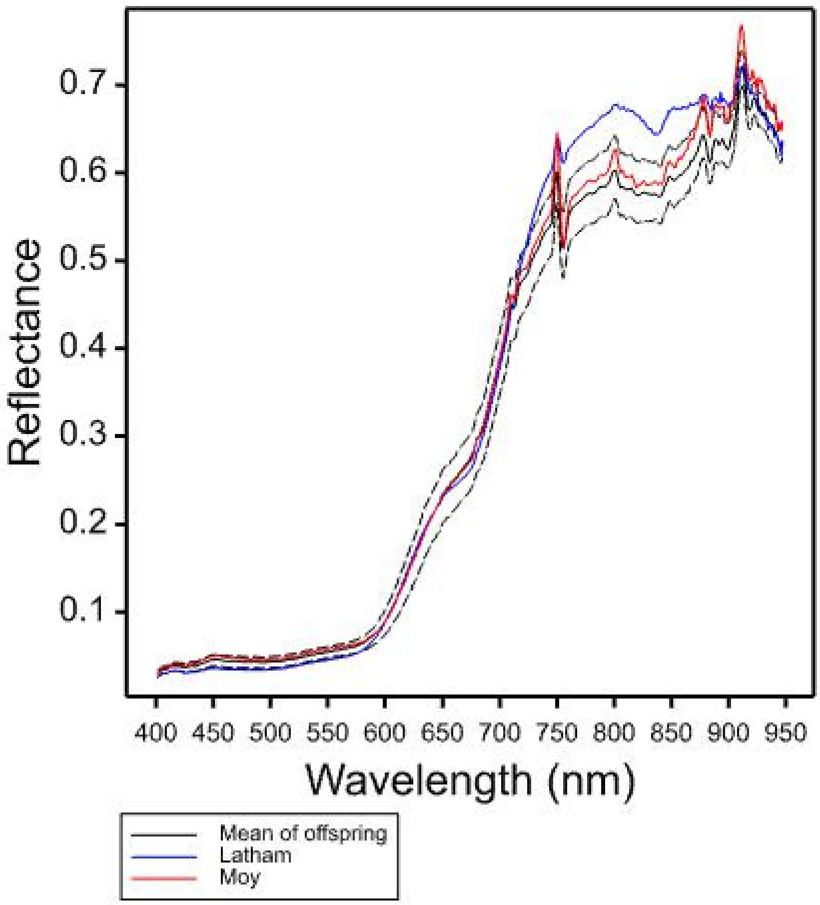
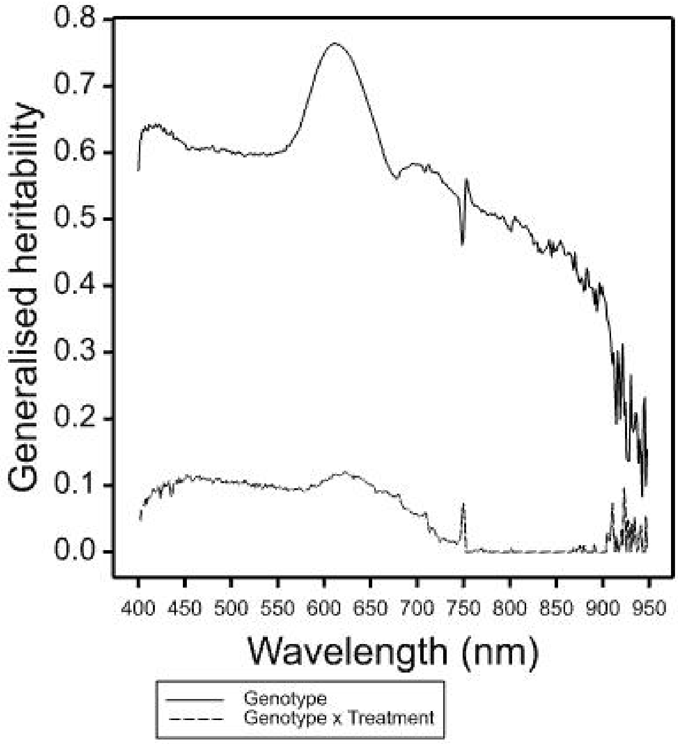


Fig. S1. Spectral reflectance profile of berries



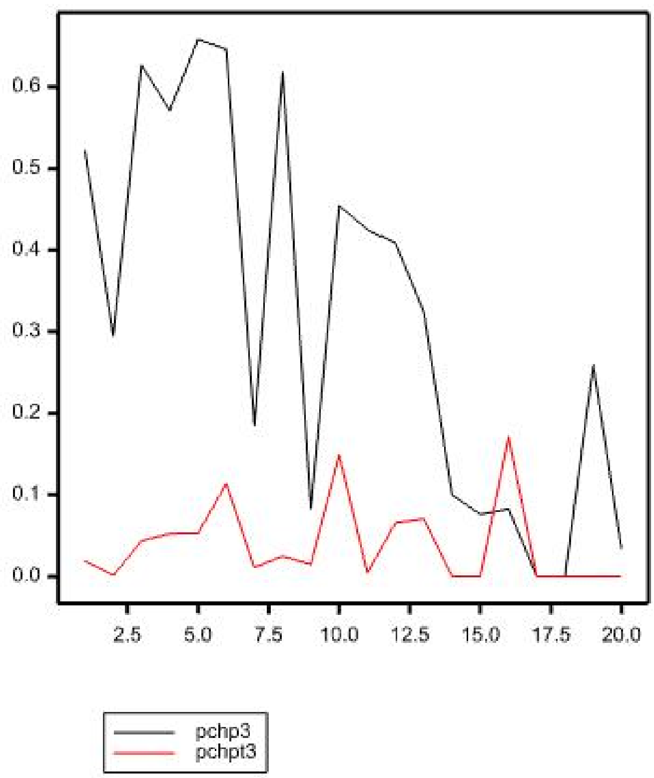


Fig. S2 Generalised heritability of spectral data collected for berries in July 2017. (a) Heritability of individual wavelengths and (b) Heritability of principal components.

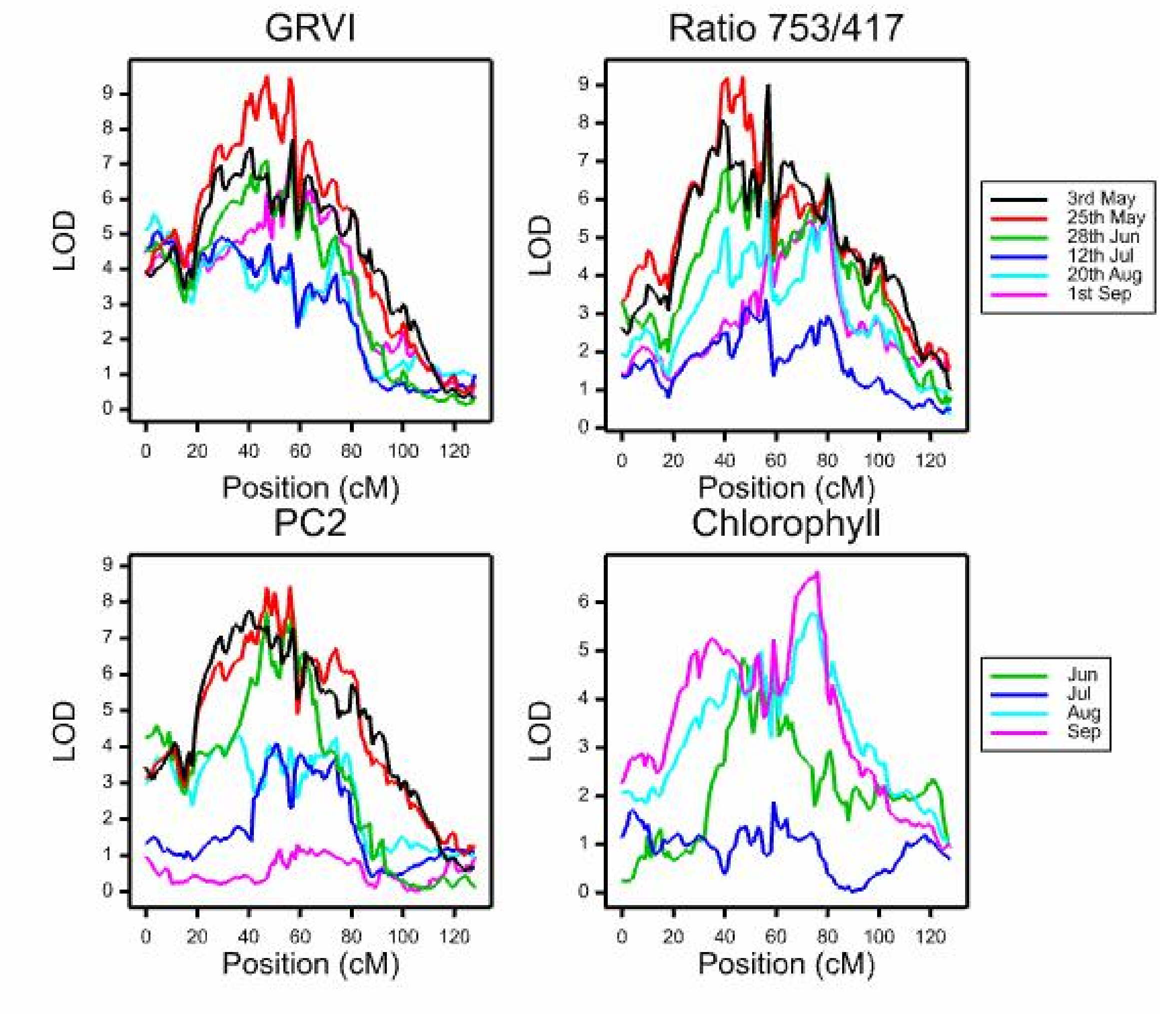


Fig. S3 Profile plots of LOD scores for four different spectral and physical traits (GRVI, 753/417, PC2 and leaf chlorophyll concentration) in linkage group 3 across the 2017 season.

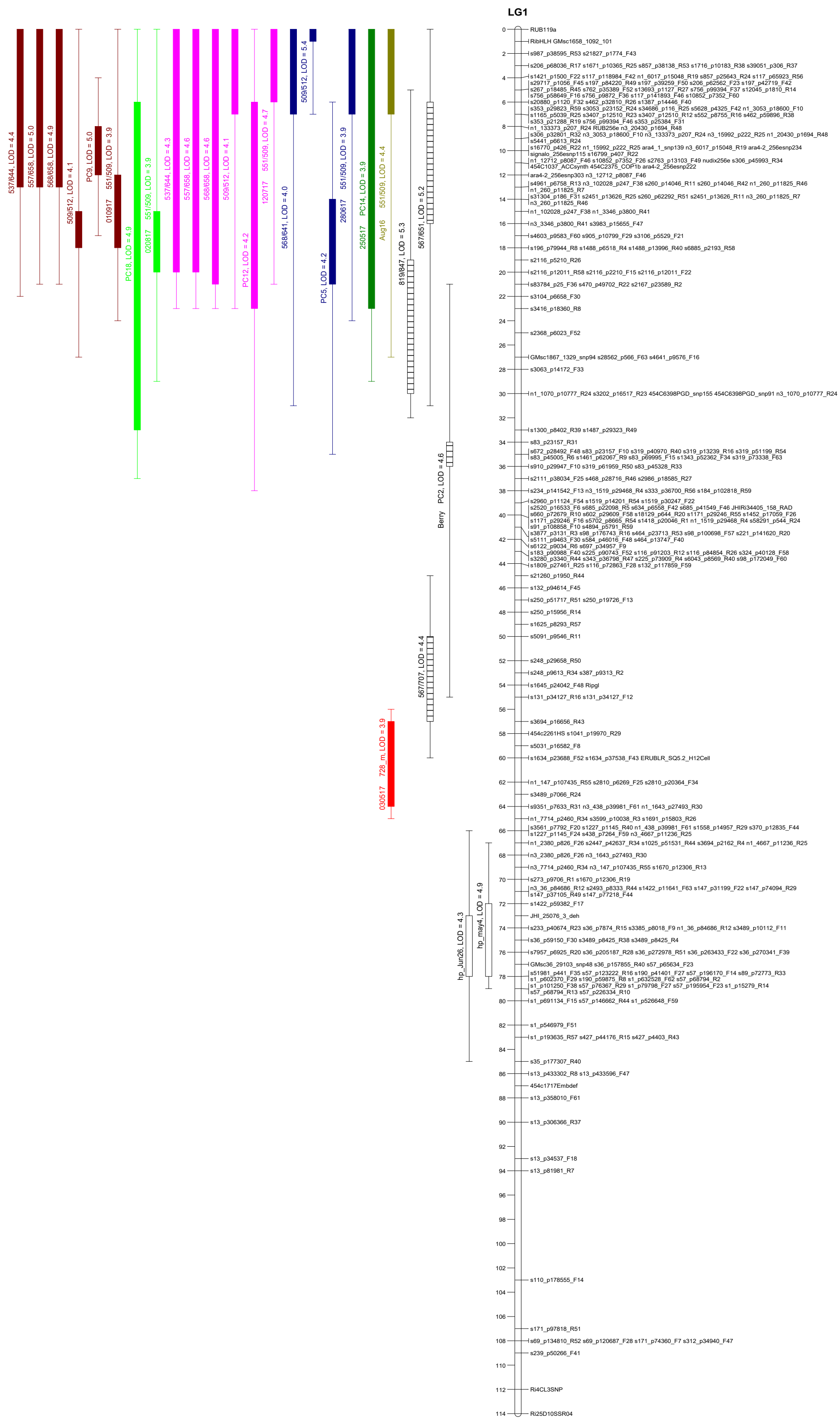


Fig. S4a. Plot showing locations of QTLs found for different spectral and physical traits in linkage group 1. The boxes represent the one-LOD support intervals and the whiskers show the two-LOD support interval (i.e. the positions where the LOD has decreased by one or two from its maximum. Data for the seven dates are distinguished by colour and shading.)

Fig. S4b. Plot showing locations of QTLs found for different spectral and physical traits in linkage group 2. The boxes represent the one-LOD support intervals and the whiskers show the two-LOD support interval (i.e. the positions where the LOD has decrease by one or two from its maximum. Data for the seven dates are distinguished by colour and shading.

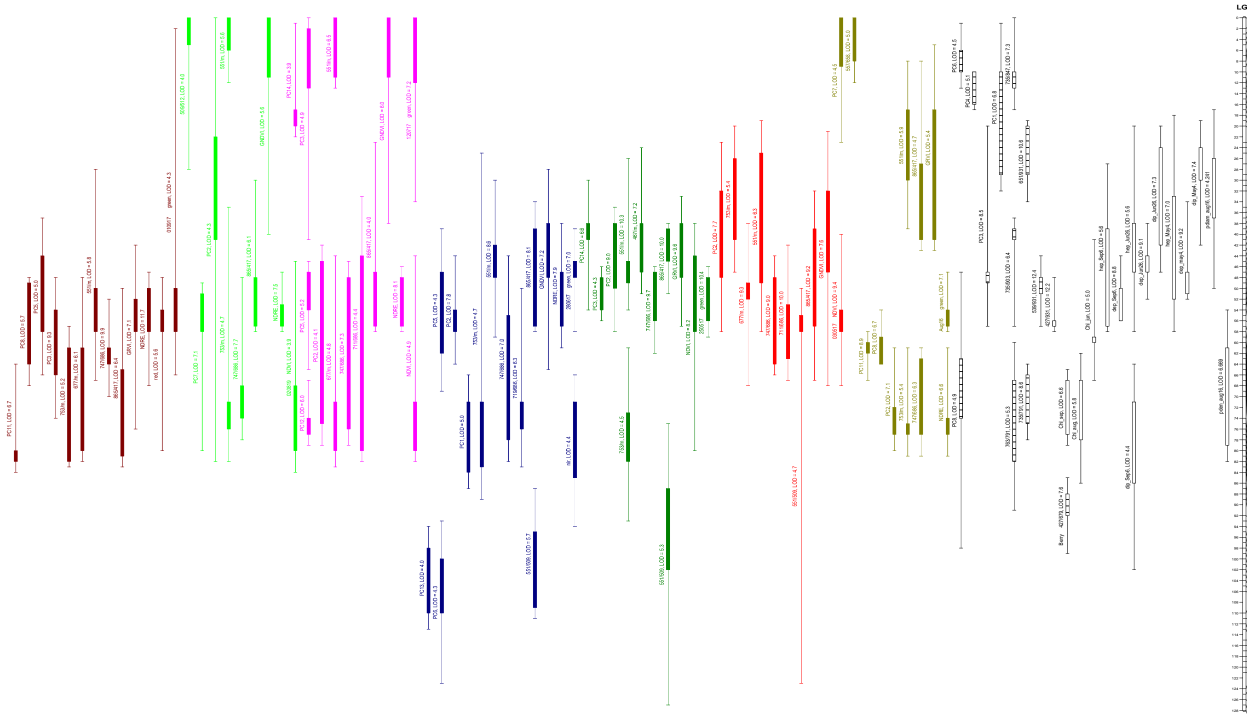


Fig. S4c. Plot showing locations of QTLs found for different spectral and physical traits in linkage group 3. The boxes represent the one-LOD support intervals and the whiskers show the two-LOD support interval (i.e. the positions where the LOD has decrease by one or two from its maximum. Data for the seven dates are distinguished by colour and shading.

Fig. S4d. Plot showing locations of QTLs found for different spectral and physical traits in linkage group 4. The boxes represent the one-LOD support intervals and the whiskers show the two-LOD support interval (i.e. the positions where the LOD has decrease by one or two from its maximum. Data for the seven dates are distinguished by colour and shading.

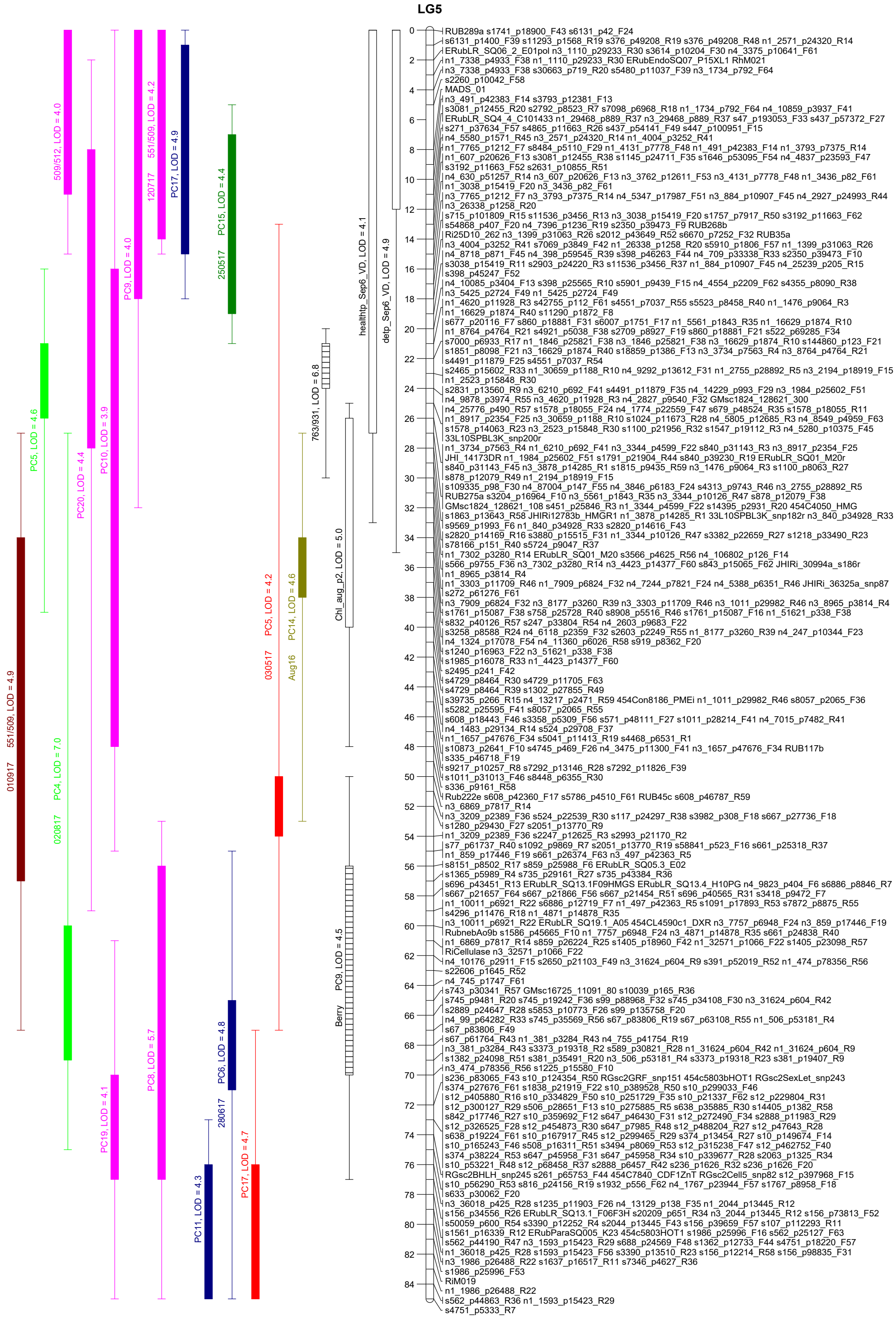


Fig. S4e. Plot showing locations of QTLs found for different spectral and physical traits in linkage group 5. The boxes represent the one-LOD support intervals and the whiskers show the two-LOD support interval (i.e. the positions where the LOD has decrease by one or two from its maximum. Data for the seven dates are distinguished by colour and shading.

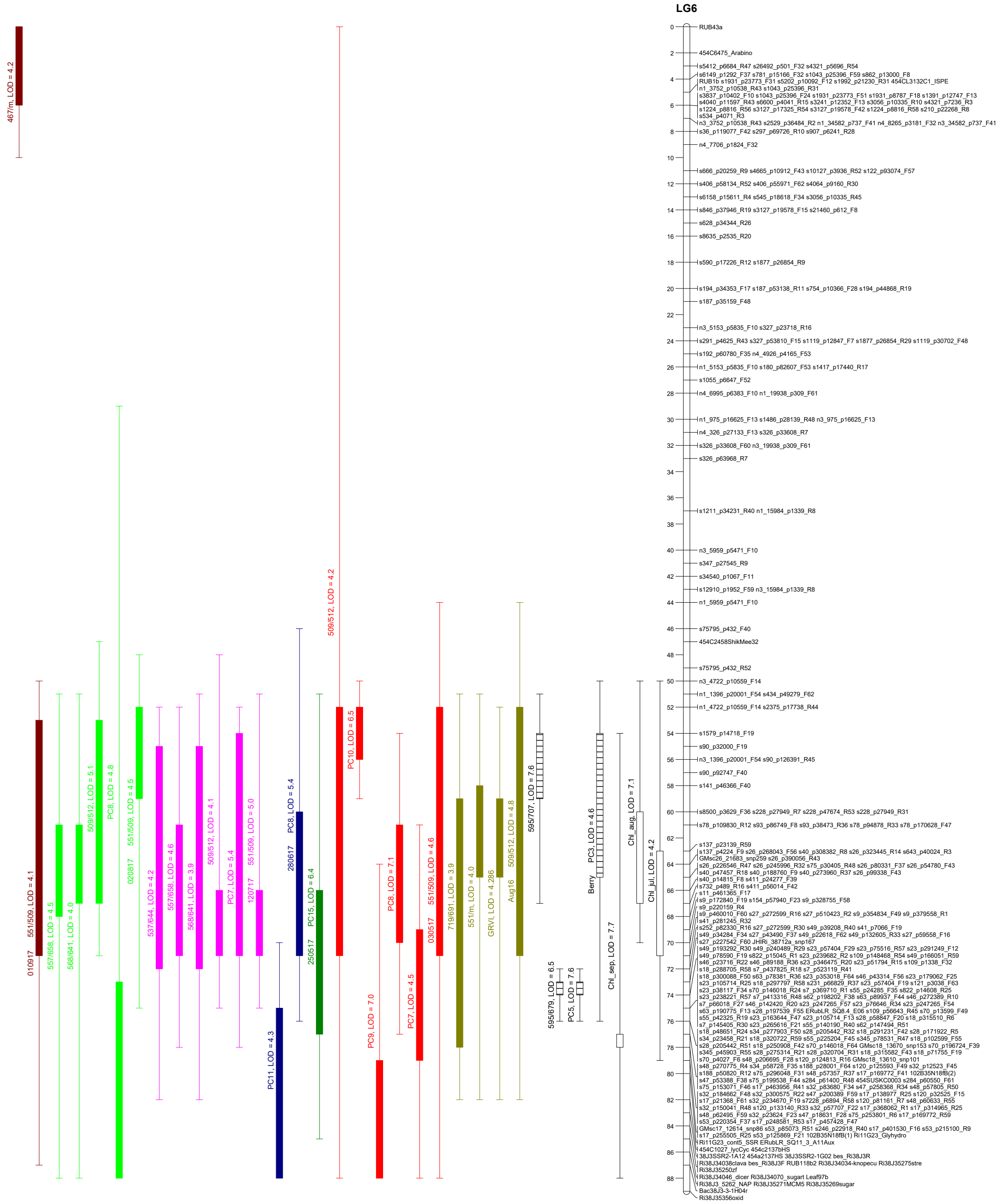


Fig. S4f. Plot showing locations of QTLs found for different spectral and physical traits in linkage group 6. The boxes represent the one-LOD support intervals and the whiskers show the two-LOD support interval (i.e. the positions where the LOD has decrease by one or two from its maximum. Data for the seven dates are distinguished by colour and shading.

Linkage Group 1

In August 2016 there was only one trait (551/509) mapping to LG1, at 6 cM (Table 3) with a LOD of 4.4, so not significant at 99% threshold. In 2017 QTLs were found in this region (1-20 cM) in June, July, August and September (Fig. S4a). The maximum LOD score for a spectral trait in June is 5.4 for a nearby ratio 509/512 at 1 cM; this trait also maps to the same region in July and September with LODs of 3.87 and 4.64 respectively. None of the physical plant scores collected in this study map onto this area of the linkage group, though previously a number of traits such as anthocyanin pigments [1,2], fruit weight and size and fruit firmness [3] and crumbly fruit [4] mapped in the 0-18 cM region. All the previously detected QTLs here are for traits relating to fruit but spectral traits are based on whole plant reflectance. This could indicate they relate to different processes in the plant or that the imaging platform is detecting differences across entire plant which will have an effect on fruit quality.

Linkage Group 2

On LG 2 (Fig. S4b) a number of regions were identified at different positions in this group. A region around 25-38 cM was identified in August 2016 and June 2017 with different spectral traits (470/512 in 2016, green in 2017). Previous work has shown that a region coinciding with the 28 cM QTL region identified here co-locates with a number of physical traits such as Gene H[5] which causes canes either to be pubescent or non-pubescent and is associated through this region with resistance to cane diseases. The genetic control of cane spines and the number of fruiting laterals also locates to this 28 cM region [6]. A region around 98-107 cM was detected in late May, June and July 2017 (PC7, PC8, PC15). As the principal components are calculated separately for each date, the difference in PC number found here is not surprising. The August measures of leaf chlorophyll concentration mapped to 98 cM, where ripening related traits have previously been mapped [7], along with lateral number [6]. The PC scores also identified a region around 65 cM in late May and June. This 65 cM region also co-locates with previously-detected ripening related traits.

Linkage Group 3

Linkage group 3 has always been the most complicated linkage group to interpret in our previous QTL studies, and this study identified LG3 as the location of many QTLs: a simplified linkage map is shown (Fig. S4c). The main peak QTL were grouped approximately into four main regions to aid interpretation: 0-11 cM, 22-29 cM, 36-41 cM, 47-81 cM.

The 0-11 cM region was detected in July and August dates in 2017 with GNDVI and 551/m appearing here. QTLs previously located here include plant traits such as bush density and overall plant density [6] as well as fruit colour[2].

The 22-29 cM region showed a number of QTLs in August 2016 but there were few QTLs detected in 2017, only bush density in May and a principal component score in August 2017. Ripening related traits have been mapped close to this region in previous studies.

The 36-41 cM region showed some QTLs for a number of spectral traits in early May 2017 as well as plant health and diameter in June. The region has potential overlap with both the previous group and the next group making interpretation of separate QTLs here challenging. This region has appeared in previous QTL studies with multiple plant architectural QTLs including spines, leaf density, bush density, leaf hairs, cane density, cane splitting, root density and diameter [6] as well as a number of ripening and fruit traits such as colour [2] and firmness [3,7,8].

The 47-81 cM region is a very significant region for every scoring with QTLs for many spectral traits across all 2017 dates and density in May, June and September, health in May and September, and leaf chlorophyll concentration in June. QTLs are seen in this region across all dates other than early May. QTLs found have here different parental effects, indicating there may well be more distinct QTL regions. Details of parental effects are available in the supplementary table. This region has previously mapped traits such as root sucker density and diameter from the mother plant [9], lateral density, height, leaf density [6] and fruit traits including firmness and ripening [3,7,8].

Given the complexity of linkage group 3 four profile plots of LOD scores from 2017 are included in the supplementary material.

Linkage Group 4

Linkage group 4 (Fig. S4d) shows spectral traits QTLs from 0-13 cM across most dates in 2017 and in August 2016. These are found for multiple spectral traits, but the significant traits differ on different dates. For example, 467/m has a QTL with LOD value of 5.77 in May 2017, no QTL is found in June or July but then a QTL reappears with a lower LOD score (4.0) in August 2017 and again in September (LOD 5.9). This could indicate it is related to plant behaviours more expressed at the start and end of season. QTLs for cane density and plant health were also found here in May, and a small QTL for cane density in September. Previous work has found QTLs in this region for leaf density, bush density and leaf hairs [10]. QTLs were also found at other positions on some dates, as shown in Fig. 8. In September there was a QTL for PC13 mapping to 24 cM and a nearby QTL for plant height was found in June.

Linkage Group 5

Linkage group 5 (Fig. S4e) shows a range of QTL positions for different dates, although some were QTLs with low LOD scores and large support intervals. In June PC17 had a QTL with a LOD score of 4.85 at 8 cM and QTLs for ten-berry weight and fruit brix values were found in same region. Previous studies have found physical traits mapping to this region, including fruit ripening [7,8], plant height and cane splitting [10]. A QTL for leaf chlorophyll concentration in September was found at 35cM. A QTL was found for 551/509 at 49 cM in September. Previous work also found a QTL for lateral length at 65 cM (Graham et al., 2014), a region where a number of spectral QTLs were found for PC scores from imaging data throughout the season, PC17 at 82 cM in May, PC6 at 67 cM in June, PC8 at 63 cM in July and PC4 at 67 cM in August.

Linkage Group 6

Linkage group 6 (Fig. S4f) showed a consistent detection of imaging QTL across all dates in a region around 66-77 cM. The most significant QTLs were generally found for PCs: PC8 with LOD 7.1 at 61 cM in early May, PC15 with LOD 6.4 at 74 cM in late May, PC8 with LOD 5.4 at 66 cM in June and PC7 with LOD 5.4 at 64 cM in July. QTLs were also found in this region with the ratio 509/512 early May, July and August although only in August did the LOD score reach the higher 99% threshold (LOD scores 4.2, 4.1 and 5.1). QTLs were also detected at this position for leaf chlorophyll concentration in July, August and September. Previous work identified QTLs for cane splitting at 69 cM [6], for root rot damage and root diameter [9], leaf density and bush density at 86 cM, and for compositional traits for berry colour and total anthocyanin contents, as well as lateral cane density around 77 cM, again coinciding with spectral traits detected from imaging data. The detection of spectral QTLs across the season corresponding to a region known to contain physical QTLs shows our spectral traits have physical significance. The fact these spectral traits are principal components shows they are possibly more complex than can be picked up with simple wavelength ratios, presenting challenges for the application of this method to practical phenotyping.

No significant QTLs were detected for LG7.

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2. McCallum S, Woodhead M, Hackett CA, Kassim A, Paterson A, Graham J. 2010. Genetic and environmental effects influencing fruit colour and QTL analysis in raspberry. *Theoretical and Applied Genetics* 121:611-27
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4. Graham J, Smith K, McCallum S, Hedley P, Cullen D, et al. 2015. Towards an understanding of the control of 'crumbly'fruit in red raspberry. *SpringerPlus* 4:223
5. Graham J, Smith K, Tierney I, MacKenzie K, Hackett C. 2006. Mapping gene H controlling cane pubescence in raspberry and its association with resistance to cane botrytis and spur blight, rust and cane spot. *Theoretical and Applied Genetics* 112:818-31
6. Graham J, Hackett C, Smith K, Karley A, Mitchell C, et al. 2014. Genetic and environmental regulation of plant architectural traits and opportunities for pest control in raspberry. *Annals of Applied Biology* 165:318-28
7. Hackett CA, Milne L, Smith K, Hedley P, Morris J, et al. 2018. Enhancement of Glen Moy x Latham raspberry linkage map using GbS to further understand control of developmental processes leading to fruit ripening. *BMC Genetics* 19:59
8. Graham J, Hackett CA, Smith K, Woodhead M, Hein I, McCallum S. 2009. Mapping QTLs for developmental traits in raspberry from bud break to ripe fruit. *Theoretical and applied genetics* 118:1143-55
9. Graham J, Hackett C, Smith K, Woodhead M, MacKenzie K, et al. 2011. Towards an understanding of the nature of resistance to Phytophthora root rot in red raspberry. *Theoretical and applied genetics* 123:585-601
10. Woodhead M, Williamson S, Smith K, McCallum S, Jennings N, et al. 2013. Identification of quantitative trait loci for cane splitting in red raspberry (Rubus idaeus). *Molecular Breeding* 31:111-22

		3rd May 2017						25th May 2017						28th June 2017						12th July 2017						2nd August 2017						1st September 2017					
LG	Position	Max LOD	Trait	Other sig wavelengths	Other sig traits	Parent		Max LOD	Trait	Other sig wavelengths	Other sig traits	Parent		Max LOD	Trait	Other sig wavelengths	Other sig traits	Parent		Max LOD	Trait	Other sig wavelengths	Other sig traits	Parent		Max LOD	Trait	Other sig wavelengths	Other sig traits	Parent		Max LOD	Trait	Other sig wavelengths	Other sig traits	Parent	
LG1	0-10cM	-						3.89 (10cM)	PC14			Latham		5.44 (1 cM)	R80/81	R61/87, r55/40, r57/93		Latham		4.73 (1cM)	R55/40	R57/93, r80/81		Latham		-						5.05 (10cM)	PC9	R57/93, R61/87, r50/88		Dominant	
LG1	16-20cM	-						-						4.19 (17cM)	PC5			Additive		4.62 (17cM)	R61/87	R50/88, PC12		Additive		4.88 (20cM)	PC18	R55/40		Additive		4.09 (16cM)	R80/81	r55/40		Latham	
LG1	74cM				Height_May (LOD 4.87, 74cM)	Dom											Height_Jun (LOD 4.26, 74cM))	Dom																			
LG2	25-38cM	-						4.50 (38cM)	R80/81			Dominant		4.75 (25cM)	green	R55/m,PC2		Latham		3.87 (31cM)	R167/7			Dominant		-						3.95 (25cM)	PC7			Latham	
LG2	62-69cM	-						4.78 (67cM)	PC14			Additive		6.65 (69cM)	PC6	PC10,PC11		Latham		-						-						-					
LG2	86-103cM	-						4.77 (107cM)	PC15			Dominant		4.93 (98cM)	PC7			Latham		5.70 (86 cM)	PC8	PC7		Latham		-			Chl_Aug (LOD 3.97,			-					
LG3	3-11cM	-						-						-						7.15 (11cM)	green	R55/m, PC3,GND VI, GRVI		Dominant		5.64 (3 cM)	R55/m	R80/81,G NDVI, GRVI		Dominant		-					
LG3	22-29cM	6.26 (29cM)	R55/m		Diam_May (LOD 7.43, 29cM)	Dominant		-						-						3.90 (18cM)	PC14			Incorporate above?		-						-					
LG3	36-41cM	7.74 (40cM)	PC2	R135/m, r127/m,GNDVI		Additive		9.95 (41cM)	R167/7	R25/m, r127/7, r135/m, PC14		Latham					diam_Jun (LOD 7.26, 41cM), health_June (LOD 5.62, 41cM)	Additive								4.32 (36cM)	PC2			Latham							
LG3	47-63cM	10.01 (57cM)	R112/103	R100/m,r167/7, r127/7,r115/105, r25/m,r55/40, r125/105,Red, NDVI, GRVI, NDRE	den_May (LOD 9.16, 50cM), health_May (LOD 7.0, 50cM)	Additive		10.42 (49cM)	green	R55/m, r125/105, r112/103, r115/105, r100/m, PC2, PC3,GRVI, GNDVI, NDVI, red, NDRE		Additive		8.64 (47cM)	R55/m	R167/7, r100/m, r125/105, r127/7, PC2,PC5,PC12,Green,GNDVI, GRVI, NDRE, red	Chl_Jun (LOD 5.01, 59cM), den_Jun (LOD 9.07, 47cM)	Latham		8.1 (56cM)	NDRE	R112/103, PC2, PC5, r167/7,r12 5/105,r11 5/105,Red		Additive		7.49 (56cM)	NDRE	R167/7, PC7		Latham		11.74 (56cM)	NDRE	PC3,PC5,P C8, r55/m, r25/m, r125/105, r115/105, GRVI, GNDVI,	den_Sep (LOD 8.77, 54cM), health_Se p (LOD 5.63, 56cM)	Additive	
LG3	72-81cM	-						4.54 (80cM)	R127/m			Additive		6.31 (74cM)	R115/105	R112/103, r25/m, r127/m, PC1,NDVI,NIR		Additive		6.05 (76cM)	PC12	R100/m, NDVI		Dominant		7.73 (73cM)	R125/105	R115/105, r127/m, r112/104, r127/7, NDVI	Chl_Aug (LOD 5.79, 74cM)	Additive		6.75 (80cM)	PC11	R100/m, r135/m, r167/7, r127/7, r112/103, r127/m, NDVI	Chl_Sep (LOD 6.65, 76cM), diam_Sep (LOD 4.42,	Additive	
LG3	97-107cM	-						5.29 (98cM)	R55/40			Additive		5.69 (97cM)	R55/40	R57/93,r118/m,PC6,PC13		Latham		-						-						-					
LG4	0-13cM	6.15 (1cM)	R80/81	R100/m, r25/m, r112/103, r55/40, r127/7, r167/7, r115/105, PC2, r57/93, PC15, NDVI	den_May (LOD 5.23, 6cM), health_May (LOD 3.87, 6cM)	Latham		-						6.18 (2cM)	PC8	PC5,PC14		Latham		4.77 (6cM)	PC5	PC9		Additive		4.16 (2 cM)	red	R25/m		Latham		5.92 (2cM)	R25/m	R125/105, r167/7, r127/7, r115/105, r100/m, PC11,red	den_Sep (LOD 3.84, 6cM)	Latham	
LG4	19-26cM	-						-						3.92 (24cM)	PC9		Ht_Jun (LOD 4.13, 26cM)	Dominant		-						4.04 (19cM)	PC7			Dominant		5.27 (25cM)	R112/103	R55/40, PC13		Additive	
LG4	44-57cM	-						4.49 (57cM)	PC2	R55/m,GRVI, GNDVI		Additive		4.64 (46cM)	PC13			Dominant		3.98 (44cM)	PC14			Dominant		-						-					
LG4	90cM	-						-						6.64 (90cM)	PC16			Additive		-						-						-					
LG5	3-17cM	-						4.42 (11cM)	PC15			Dominant		4.85 (8cM)	PC17			Dominant		4.42 (13cM)	PC20	R80/81, r55/40, PC9		Additive		-						-					
LG5	22-41cM	-						-						-						3.92 (41 cM)	PC10			Additive		4.55 (22cM)	PC5		Chl_Aug (LOD 5.03,	Additive		4.86 (49cM)	R55/40				
LG5	51-82cM	4.74 (82cM)	PC17	PC5		Dominant		-						4.85 (67cM)	PC6	PC11		Moy		5.67 (63cM)	PC8	PC19		Moy		7.00 (67cM)	PC4			Additive							
LG6	3cM																															4.16	R25/m	R127/7			Latham
LG6	55-88cM	7.06 (61cM)	PC8	PC9, PC7, PC10, r55/40, r80/81		Latham		6.43 (74cM)	PC15			Latham		5.37 (66cM)	PC8	PC11		Latham		5.44 (64cM)	PC7	R55/40, r57/93, R50/88, R80/81, R61/87	Chl_Jul (LOD 4.16, 67cM)			5.06 (55cM)	R80/81	R55/40,r57/93,r61/87,PC8	Chl_Aug (LOD 7.12, 64cM)	Latham		4.07 (64cM)	R55/40		Chl_Sep (LOD 7.7, 77cM)		Latham
LG7	None							None																		None						None					