Journal of Horticulture
ISSN: 2376-0354
OPEN ACCESS Freely available online
Research

Developmental QTL in A Red Raspberry Primocane X Biennial Raspberry Population That Exhibit Primocane Fruiting

Julie Graham*, Kay Smith, Katrin MacKenzie, Linda Milne, Nikki Jennings, Brezo Mateos, Christine Hackett

The James Hutton Institute, Invergowrie, Scotland

ABSTRACT

The changing climate is altering timings of key fruit developmental and ripening processes and increasing the occurrence of fruit defects. This work aimed to expand the knowledge generated previously on the genetic control of the ripening process using a biennial x biennial raspberry F1 population (Latham (L) x Glen Moy (GM) (LxGM)), in this case by examining development in a biennial x primocane F1 population (Glen Fyne (GF) x Autumn Treasure (AT) (GFxAT)). The aim was to identify Quantitative trait loci (QTL) and genome locations associated with the process of development from flower bud swell to ripe fruit, to understand how developmental control in this population differs from that in the LxGM population previously studied. The progeny from this biennial x primocane population all exhibited primocane fruiting completing their lifecycle in a single season. QTL associated with development from bud break to ripe fruit were identified. These QTL differed from developmental QTL for similar developmental stages previously identified on fruiting canes (second year canes) in the LxGM population suggesting control of development differs in different populations. Some insight into gene content in QTL regions is presented.

Keywords: Raspberry; Primocane biennial; Development; QTL.

INTRODUCTION

Environmental changes are impacting developmental processes which ultimately affect yield. Higher winter temperatures are leading to uneven bud break in raspberry and a shift in flowering time [1], also seen in other crops in response to climate change [2,3]. The transition to flowering is regulated by multiple environmental and internal cues which affect timing, as flowering at appropriate times ensures best use of the available growing season and is therefore relevant to yield and quality.

Raspberry is a temperate species that bears short lived woody shoots on a long lived perennial root system bearing juvenile and mature shoots (canes) simultaneously on an individual plant. In biennial fruiting cultivars (also known as floricane or summer fruiting) the canes have a two year life cycle. In contrast, primocane cultivars (also known as annual or autumn fruiting) complete the cycle of vegetative growth, flowering, and fruiting in a single season. Haskell and Lewis [4] regarded primocane fruiting as a discrete character which could be considered characteristic of all or nearly all a plant's canes and postulated that it was controlled by a major gene. This interpretation is not generally supported. Keep [4] found that autumn fruiting was a continuous varying character determined quantitatively by genes acting in an additive or complementary way. The growth cycle of raspberry has been described in detail by Jennings [5] who also considered perennial and biennial fruiting as a continuous response to a range of day length/temperatures. In effect primocane fruiting genotypes are considered day length and temperature neutral [5] since they initiate their flowers in long days and high temperatures in contrast to the short day and low temperatures required for main season raspberries. In biennial fruiting cultivars the dormancy requirement defines the two year fruiting mode and thus chilling requirements, and day length are key to fruiting behavior [6]. Climate neutral traits may become

Correspondence to: Julie Graham, The James Hutton Institute, Errol Road,a Invergowrie, Dundee, Scotland, DD25DA

Tel: +441382568739, Email: Julie.Graham@Hutton.ac.uk

Received: 30-May-2022, **Manuscript No:** Horticulture- 22-17681; **Editor assigned:** 01-June-2022, **PreQC No:** Horticulture- 22-17681 (PQ); **Reviewed:** 15-June-2022, QC No: Horticulture- 22-17681; **Revised:** 20-June-2022, **Manuscript No**: Horticulture- 22-17681 (R); **Published:** 27-June-2022, **DOI:** 10.35248/2376-0354.22.9.1000308

Citation: Julie Graham, Kay Smith, Katrin MacKenzie, Linda Milne, Nikki Jennings, Brezo Mateos, et al. (2022) Developmental QTL in A Red Raspberry Primocane X Biennial Raspberry Population That Exhibit Primocane Fruiting. J Hortic 9:308.

more important as seasonal fluctuations continue and become more unpredictable with effects already apparent with irregular and unpredictable bud break in biennial cultivars. For reviews of the developmental transitions to flowering in raspberry see Kurokura [7] and Graham and Simpson [8].

Photoperiod has a major role in flowering time in many plants perceived in the leaves with a signal or 'florigen' which integrates photoperiod and temperature signals and transmits the command to flower [9]. Plants keep track of the photoperiod with an endogenous clock [10,11]. The role of the clock in flowering time control in Arabidopsis was demonstrated by QTL mapping of flowering, which identified clock related genes [12]. Activity of CONSTANS (CO) the key component in leaves of the photoperiodic pathway is controlled by the circadian clock. At the appropriate time, CO activates transcription of FLOWERING LOCUS T (FT) which moves to the shoot apical meristem where it interacts with bZIP transcription factors [13] activating floral integrator genes including SUPPRESSOR OF CONSTANS OVEREXPRESSION1 and APETALA1 thus inducing a cascade of downstream genes leading to flowering.

The vernalisation pathway also plays a key role in regulating development of cold sensitive floral organs. In Arabidopsis, a MADS box transcription factor FLOWERING LOCUS C (FLC) plays a central role in repression of flowering before vernalization [14]. FLC is upregulated by FRIGIDA (FRI) in non-vernalized plants [15]. FLC delays flowering by binding to the regulatory regions of floral activators including FT, SOC1 and bZIP FD [13,16,17]. After vernalization FLC remains silenced under warm conditions enabling upregulation of FT and SOC1. In the Shoot apical meristem (SAM) the FT protein forms a heterodimer with FD which promotes flowering by activating APETALA 1.

In a biennial raspberry population derived from a cross between the cultivars Latham and Glen Moy (LxGM) [18], QTL were identified across six of the seven raspberry linkage groups (LGs 2-7) for the timings across development to individual developmental stages from bud break to ripe fruit and for principal coordinate analysis (PCO) that summarized the developmental process. The data were subsequently re-analysed on a more marker dense genotyping by sequencing (GbS) map linked to genome scaffolds, which provided greater insight into the genes regulating key steps in the developmental process [19]. The range of QTL for different stages of fruit development identified genetic regions rich in genes relevant to development, flowering, and the process of ripening (see [19] for the full list of genes identified within QTL). Briefly, across the linkage groups genes identified include a DIVIA like MYB transcription factor (WEREWOLF) on LG 2 [20], which could be controlling flowering time, as this has been shown to be a posttranscriptional regulator of FT, a key floral regulator [21]. A MADS domain transcription factor, AGAMOUS, a pollen expressed transcription factor; PTF2 and a range of other genes were also identified linked to QTL on LG 2. LG 3 had a strong effect on ripening with a broad range of significant markers identified suggesting more than one QTL was involved. FT, transport of FT (FTIP1), a gene regulating levels of FLC (EFL7) and CONSTANS-LIKE 9 (COL9) regulating CO, FT, SOC1 and SPT16 which binds to the FLC promoter and a bZIP were identified in QTL on LG3. Also associated with the QTL is RiMYB known to control phenylpropanoid metabolism including anthocyanin accumulation in fruit [22,23] but also with important roles in many other aspects of plant development including trichome development, signal transduction, disease resistance and cell division [24]. RiMADS_01 was identified on raspberry LG5 [18] as a potential candidate affecting vernalization and is close to a QTL for green fruit and fruit set identified in the 2018 study [19] as well as QTLs for several PCO scores that summarise the speed of ripening. RiMADS_01 is similar to SHORT VEGETATIVE PHASE protein (SVP) modulating the timing of the developmental transition to flowering phases in response to temperature. In the 2009 paper [18], in colder seasons RiMADS_01 was associated with earlier flowering. Close to RiMADS_01 is a region which included a range of genes regulating embryo development, flower development, meristem development, cell development and photoperiodism, including ALTERED MERISTEM PROGRAM 1 (AMP1), FLOWERING PROMOTING FACTOR 1 (ATFPF1) and REDUCED VERNALISATION RESPONSE (VRN1). On LG 6 the genes identified mainly had roles in embryo development during fruit set.

The 2018 study [19] also identified a range of auxin signaling and response gene homologs within the QTL including ATCUL1, TOPLESS, NAC17, 12A, ARF6, ARF17, and ATAVP1. Other genes involved in ethylene synthesis, activation and signalling were identified within multiple QTL across LG3, LG5 and LG7. Raspberry is non-climacteric, but ethylene formation may have a minor role in raspberries that may be co-ordinated with auxin and ABA formation as part of the mechanism that regulates timing of ripening in different fruit species.

The aim of this work was to expand our knowledge of the genetic control of the bud swell to fruit ripening process, by examining the timing of developmental transitions to ripe fruit in a biennial x primocane F1 population in the same way as the LxGM population previously studied. We aimed to determine if the progeny exhibited both flowering modes, which if any was dominant, and to examine QTL and genome locations associated with development to gain an understanding of how control in this population might differ from the biennial control previously observed in the LxGM population.

This required constructing a basic framework map for the GFxAT cross using markers from the biennial x biennial LxGM map to allow comparisons of QTL to be made and phenotyping as previously used in the LxGM cross. This would provide information on how this population develops in terms of QTL identified and how these compared with the LxGM population previously characterized.

To achieve more adapted varieties, we need to determine and then understand the developmental control in the different flowering types and crosses between them. If the F1s flower in spring, it would suggest that biennial flowering is recessive. However, if the F1s do not flower until later in the year and do not set fruit until the following year, it will reveal that biennial flowering is dominant. Other scenarios in the population may include progeny which express a 1:1, or a continuum of flowering between the two parental types. In a previous study on tetraploid blackberry, the primocane fruiting trait was recessive in the cross studied [25]; however, we do not know if this is the same for red raspberry or if it will be in the case in all populations. The Castro study [25] examined the flowering type but did not examine the development process across ripening.

MATERIALS AND METHODS

Population development

In order to identify key loci controlling development to ripe fruit, 181 full-sib F1 individuals were generated from a cross between varieties Autumn Treasure (AT), a primocane variety bred by the East Malling (NIAB/EMR) raspberry breeding consortium (provided by RW Walpole Ltd propagators) with excellent architectural traits such as ability to heal cane wounds and a vigorous root system and Glen Fyne (GF) bred at the James Hutton Institute by the raspberry breeding consortium (provided from the James Hutton Institute high health facility), a biennial variety with excellent fruit quality. This segregating F1 population (GFxAT) (developed by N. Jennings the James Hutton Institute consortium raspberry breeder) was planted in the field in three plant plots across three randomised replicates (9 clonal plants per each of the 181 genotypes). After a full season to allow the plants to establish, the population was phenotyped for developmental processes in the following two seasons.

Phenotyping

Phenotyping was carried out as previously described [18]. Briefly, the plants were scored for the latest developmental stage throughout the period from bud swell to fruit development/ripening process in two years (s1 and s2) after an initial season (s0) to allow the plants to establish using a 0-7 scale as follows:

0: Tightly closed buds

1: Closed bud swell

2: >10% open flowers

3: >10% immature fruit set

4: Green fruit

5: Green/red fruit

6: Red fruit

7: Over ripe fruit

Examples of developmental scores across flowering through to ripe fruit are given in Table 1 with P denoting the stage of development on the primocane.

The cane type (primocane) and date scored (numbered sequentially) representing the mean developmental stage across the progeny, became the trait name used for QTL analysis in seasons 1 and 2 (Table 2) (eg. P2_s1_would be the average stage of development reached on the primocanes (P) from the second date analysed (2) being 18th August, in season 1 (s1) for QTL analysis and in this case represented bud swell as the mean stage reached across progeny.

Table 1: Summary of developmental trait data for parents and progeny recorded over the two seasons.

Season	Primocane stage and date scored (average stage represented in offspring)	Treasure	Treasure	Fyne	Fyne	Offspring	Offspring	SD	G_herit
		Mean	Median	Mean	Median	Mean	Median		
1	P1_21 July (closed buds)	0.83	1	0	0	0.05	0	0.22	0.6
	P2_18 August (bud swell)	3.83	4	0	0	1.1	1	0.92	0.76
	P3_25 August (bud break)	5.17	5.5	0	0	1.56	1	1.07	0.78
	P4_1 September (flowering)	6	6	0	0	2.15	2	1.27	0.8
	P5_9 September (fruit set)	7	7	0.8	0	3.03	3	1.33	0.76
	P6_22 Sept. (green fruit)	7	7	1.2	1	4.04	4	1.12	0.76
	P7_8 October (green/red)	7	7	1.6	1	5.36	5	1.45	0.81
	P8_15 October (ripe red fruit)	7	7	2.4	3	6.06	7	1.27	0.79
2	P1_23 July (closed buds)	1	1	0	0	0.14	0	0.37	0.51
	P2_30 July (bud swell)	2.17	2	0	0	0.65	0	0.99	0.06
	P3_7 August (flowering)	4	4	0	0	1.92	2	1.73	0.15
	P4_19 August (fruit set)	5	5	0	0	2.67	4	1.98	0.16
	P5_28 August (green fruit)	5.8	6	0	0	3.53	4	2.37	0.22

Table 2: QTL data across linkage groups with marker and genotype means in scoring season 1.

Trait (see Table 1 for details)	LG	Position	Nearest Locus	LOD	AC	AD	BC	BD	RMS	% Explained
P2_s1 (bud swell)	1	46.6	LG4sc111b	4.1	1.57	0.94	0.9	0.91	0.54	14.4
P1_s1 (closed buds)	1	59.1	GMsc_49424_135_Aq	4.6	0.17	0	0.02	0.02	0.03	15.4
P_PCO2_s1	2	0	GMsc1970_snp176	7.3	0.07	0.06	0.03	-0.1	0.02	24.9
P_PCO3_s1	2	0	GMsc1970_snp176	5.5	-0.01	0.02	-0.06	0.04	0.01	15
P_PCO1_s1	2	22.7	GMsc592_39439_snp331	6.5	-0.16	-0.02	0.09	0.13	0.06	16.8
P1_s1 (closed buds)	2	27.2	GMsc592_39439_snp188	4.6	0.17	0.03	0	0.03	0.03	11.7
P2_s1 (bud swell)	2	26.7	GMsc592_39439_snp188	5.4	1.59	1.18	0.77	0.9	0.54	14.1
P3_s1 (bud break)	2	26.7	GMsc592_39439_snp188	5.8	2.19	1.53	1.2	1.41	0.71	15.1
P4_s1 (flowering)	2	24.7	GMsc592_39439_snp188	5	2.84	2.21	1.78	1.84	1.04	13.4
P5_s1 (fruit set)	2	22.7	GMsc592_39439_snp331	4.7	3.66	3.14	2.67	2.67	1.09	12.5
P6_s1 (green fruit)	2	22.7	GMsc592_39439_snp331	4.9	4.57	4.13	3.7	3.76	0.74	13
P7_s1 (green/red fruit)	2	18	GMsc349b	5.7	5.91	5.61	5.19	4.66	1.31	14.4
P8_s1 (ripe fruit)	2	17	GMsc349b	5.8	6.47	6.36	5.88	5.46	0.99	14.2
P_PCO2_s1	2	97.5	GMsc590_cr	5.4	-0.11	0.04	0.05	0.06	0.02	22.8
P1_s1 (closed buds)	2	97.5	GMsc590_cr	4.9	0.2	0.01	0.01	0	0.03	19.2
P_PCO1_s1	4	5.3	GMsc213_47434_MYB4	7.1	-0.17	0.01	0.09	0.09	0.06	18.8
P2_s1 (bud swell)	4	5.3	GMsc213_47434_MYB4	6.4	1.64	1.06	0.78	0.91	0.52	17.9
P3_s1 (bud break)	4	5.3	GMsc213_47434_MYB4	7.1	2.2	1.51	1.25	1.27	0.67	19.2
P4_s1 (flowering)	4	5.3	GMsc213_47434_MYB4	6.8	2.92	1.99	1.83	1.86	0.98	18.2
P5_s1 (fruit set)	4	5.3	GMsc213_47434_MYB4	6.3	3.77	3.04	2.72	2.65	1.04	16.9
P6_s1 (green fruit)	4	5.3	GMsc213_47434_MYB4	6.4	4.63	4.07	3.76	3.71	0.71	17.1
P7_s1 (green/red fruit)	4	5.3	GMsc213_47434_MYB4	4.3	6.01	5.4	5.04	5.01	1.35	11.4
P8_s1 (ripe fruit)	4	5.3	GMsc213_47434_MYB4	4.5	6.59	6.21	5.75	5.73	1.02	11.6
P2_s1 (bud swell)	4	77.2	FruitG7	5.3	1.66	0.93	0.97	0.95	0.54	14.8
P3_s1 (bud break)	4	76.4	FruitG7	4.6	2.2	1.34	1.38	1.46	0.71	14.6
P1_s1 (closed buds)	4	105.8	GMsc2823_23204_y	5.4	0.01	0.01	0.01	0.19	0.03	18.5

P1_s1 (closed buds)	5	100.2	GMsc14000_RR	4.9	0	0.02	0.01	0.17	0.03	13.8
P_PCO1_s1	6	51.3	GMsc18_13610_ snp113	6.7	-0.01	0.08	-0.19	0.1	0.06	18.7
P2_s1 (bud swell)	6	50.3	GMsc18_13610_ snp113	6.2	0.95	0.96	1.75	0.86	0.51	20.3
P3_s1 (bud break)	6	50.3	GMsc18_13610_ snp113	6.4	1.56	1.32	2.26	1.27	0.68	18.8
P4_s1 (flowering)	6	51.3	GMsc18_13610_ snp113	5.2	2.2	1.89	2.9	1.85	1.02	14.8
P5_s1 (fruit set)	6	52.3	GMsc18_13610_ snp113	8.2	3.12	2.76	3.93	2.59	0.98	21.3
P6_s1 (green fruit)	6	54	LG6sc23b	6.1	4.2	3.83	4.63	3.72	0.72	15.1
P7_s1 (green/red fruit)	6	52.3	GMsc18_13610_ snp113	5.6	5.53	5.14	6.14	4.89	1.3	14.9

Map construction

DNA was extracted from parents and progeny as previously described [26]. A range of Simple

sequence repeats (SSR) and Single nucleotide polymorphism (SNP) markers from LxGM linkage maps [26-35] were tested on the GFxAT population to allow the LxGM and GFxAT maps to be comparable and enable linkage to the GM genome sequence. These are presented in Supplementary Table 1 for ease of reference.

Primers to either SNPs or SSRs from the GbS Latham x Glen Moy map [19] were generated using primer 3 or Kompetitive Allele Specific PCR (KASP) by Design (KBiosciences) (Supplementary Tables 1 and 2). SNPs from the GbS scaffolds were identified using the Glen Moy genome sequence and by mapping RNA-seq data from 22 raspberry varieties onto the draft genome sequence (JHI resource, [36]) using Bowtie2 and the individual BAM files were merged into one. SNPs were discovered using freebayes using settings"~ploidy 2~no-population-priors~min-alternate-count 5~min-alternate-fraction 0.2". Poor quality SNPs were filtered using a custom Java program.

Polymerase chain reaction (PCR) products were run on the ABI 3730 capillary sequencer (Applied Biosystems, Foster City, CA, USA) using ROX500 (Applied Biosystems) as an internal size standard. Size polymorphisms were analysed using Genemapper. v5 (Applied Biosystems). SNPs were identified by sequencing, (Big Dye Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems) run on ABI 3730 capillary sequencer and analysed using Sequencher v5.2.4. KASPar assays were carried out with 2xKASPar v4.0 Reagent (KBioscience) and 40 ng of template DNA. These were run and analysed on 7500 Fast Real Time System (Applied Biosystems). PCR conditions for KASPar were as follows: 2 minutes @20^{O}C (Pre PCR read), 15 minutes @94 ^{O}C, 20 seconds @94^{O}C, 1 minute @62^{O}C (decreasing by 0.7^{O}C per cycle), 10 cycles, 20 seconds @94^{O}C, 1 minute @55 ^{O}C, 32 cycles, 2 minutes @20^{O}C (Post-PCR read).

Linkage analysis

The linkage map was constructed using the Join Map 4.1 software [37]. A total of 267 markers segregated in the population. A significant number of markers tested were monomorphic highlighting the highly bred nature of the parents. Segregation of the markers confirmed all progeny were from true seed generated from the ATxGF cross. Any marker for which more than 20% of the population had missing scores was excluded from the analysis. Markers were clustered into separate linkage groups based on their independence Logarithm of the odds (LOD) score and then ordered within linkage groups using Join Map's regression analysis ordering. Markers with a poor goodness of fit measure when placed on the map or showing highly significant distortion from the expected segregation ratio were excluded from the analysis.

QTL analysis

The phenotypic data consists of repeated ordinal scores on each plot. These can be analyzed separately (Table 1) or combined into measures of overall ripening (Table 3) [18]. Here, a principal coordinate analysis (PCO) was used to summarize the ripening profile into independent PCO scores. The similarity between plots was calculated using the city block measure of similarity and then the first five PCO scores were calculated. This was carried out for the primocane scores for each year separately (as the number of scoring occasions differed between years). Correlations between the principal coordinates and the original scores were examined to see how these were related and to interpret the PCOs.

Table 3: Summary of PCO statistics.

Season	Fruiting Type	PCO (explanation of what high scores relate to)	% variability	G_heritability
S1	Primocane	1 (average rate of development)	42.6	0.88
	Primocane	2 (start slow)	14.4	0.73
	Primocane	3 (rapid development)	5.9	0.66
	Primocane	4	4.1	0.36
	Primocane	5	2.9	0.1
S2	Primocane	1 (average rate of development)	54.5	0.24
	Primocane	2 (start slow)	13.3	0.15
	Primocane	3 (rapid development)	6.7	0.18
	Primocane	4	5	0.28
	Primocane	5	3.9	0.08

A general linear model of the individual ripening scores and the first five PCO scores from each set of measurements was used to estimate the genetic and environmental components of variance, and to predict the mean trait scores for each genotype. For some measurements preliminary analysis showed spatial variation among the rows within each replicate, and so row was also included as a factor in the linear model. The estimated genotype means for the offspring were then used as phenotypic traits for QTL mapping. A mixed model was used to estimate the generalized heritability for each trait [38]. This was calculated using GenStat 20 (GenStat for Windows 20th Edition 2019, VSN International, Hemel Hempstead, UK, and GenStat.co.uk) and its VHERITABILITY procedure.

QTL mapping was carried out using the Map QTL 5 software [39]. The Kruskal-Wallis test implemented in Map QTL was used as a preliminary test to identify regions of the genome that were linked to each of the individual scores and principal coordinates, and whether the phenotype was affected by alleles from one parent or both. Interval mapping was then carried out using Map QTL. A LOD threshold for interval mapping was conducted using a permutation test of 1000 permutations of each of the PCO scores from the field and polytunnel, and then taking the mean of the 95% threshold from each trait to use as a common threshold. The permutation test was conducted for each season separately as there was more missing trait data in the second season of scoring.

RESULTS AND DISCUSSION

Phenotype data

After a full growing season to allow the plants to establish, the plants were scored for development in the two following seasons. Autumn Treasure and Glen Fyne behaved as expected. Autumn Treasure (primocane) showed movement in the buds by mid-July and ripe fruit on the primocanes by the beginning of September. Glen Fyne (main season) showed a few swelling/open buds at the top of the primocane by mid-October however these did not develop further. The progeny from this cross, although showing variation in the rate at which they progressed through the developmental stages (with several developmental QTL identified, most of which showed significant effects from both parents), all completed their lifecycle in one season. On average across the progeny the buds showed movement by mid-August and ripe fruit by early to mid-October around 5 weeks behind Autumn Treasure. Thus, in this cross the primocane fruiting trait was dominantly expressed and did not segregate, therefore primocane fruiting as a trait could not be mapped. From the data here it may suggest two hypotheses. The first being that the primocane fruiting trait is consistent with a single gene controlling whether primocane-type behaviour occurs, while multiple QTL control the speed of the developmental processes. If this were the case, it could be hypothesised that Autumn Treasure must be homozygous at that locus and crossing the offspring to Glen Fyne would be a good test of this hypothesis. Alternatively, it could be that multiple factors that control the timing and speed of the developmental processes are responsible for regulating fruiting behaviour. As alleles from both parents contribute to most of the QTL it may suggest the second option with multiple factors is most likely.

By the end of season one of phenotyping, plants in 30 plots were scored as dead. By the end of season two of phenotyping (year 3 of planting), the number of dead plants had increased to 108 due to Phytophthora root rot in the field plots and hence less robust data was available from season two and this is reflected in the lack of robust QTL identified. However, the QTL identified in season 1 of scoring were significant and thus allowed comparison with the developmental process in the LxGM biennial cross which was the objective of the study.

Table 1 shows the mean and median development stage scored for the parents in each year and the mean, median and standard deviation for the offspring across a selection of the dates, together

with the generalised heritability. It also shows the percentage variance explained by each PCO score and their generalised heritability (Table 3). The heritabilities are highest in the first year of scoring but much lower in the second due to reduced plant numbers. The first principal coordinates (PCO1) for each set shows positive correlations with all the individual measurements and can be thought of as an average rate of development. The variability was 43% in season one primocanes, and 55% in season two primocanes, and the generalised heritabilities are 0.88, and 0.24 respectively. The heritabilities of the other principal coordinate scores are generally lower except for PCO2 and PCO3 in season one. PCO2 for this set has significant positive correlations with the later scores (October) and negative correlations with the earlier scores (July/August), so PCO2 will have largest values for genotypes that started developing slowly and then developed rapidly towards the end of the season. PCO3 for this set has significant positive correlations with the July and mid-August scores so will have largest values for genotypes that develop rapidly. In season 2, progress through the developmental stages occurred earlier than in season 1 (Table 1) highlighting the impact of the environmental factors such as temperature from the different seasons.

Linkage map construction

Many markers were tested and 70% of these were found to be monomorphic within and between parents highlighting the highly bred nature of the parents. Those markers that were showing heterozygosity were mapped as described.

The markers clustered clearly into linkage groups. Five groups were separated by Join Map at a LOD of 4.0 and the final group separated into two at a LOD of 5.0. The groups varied in size from 7 to 39 markers, and in length from 34.6 cM to 107.6 cM. Summary statistics are given in Table 4. There are markers in each group that are also on the Latham x Glen Moy (LxGM) map, and these were used to match the maps and orientate the linkage groups to correspond to the published Latham x Glen Moy maps [18,19,27,30-32,34-35,40-45]. All groups had markers that were heterozygous in Autumn Treasure only, Glen Fyne only and in both parents. Most of the markers that are heterozygous in both parents have three or four alleles and so identify all four offspring genotype classes, meaning that despite their low number of markers, these maps are informative regarding the offspring genotypes for QTL mapping.

Table 4: Linkage group summary table and LG nomenclature for strawberry.

Linkage Group (LG)	Strawberry LG Nomenclature	No. Markers	Autumn Treasure	Glen Fyne	Both		Length (cM)
(LxGM nomenclature)	Bushakra et al [47]		(ab x aa)	(aa x ab)	(ab x cd) or (ab x ac)	(ab x ab)	
1	FLGVII	21	2	15	4	0	71.8
2	FLGIII	32	11	10	7	4	104.2
3	FLGVI	31	18	7	1	5	107.6
4	FLGII	32	13	10	8	1	105.8
5	FLGV	39	12	15	8	4	100.1
6	FLGI	34	8	9	13	4	82.5
7		7	1	3	3	0	34.6
Total		196	65	69	44	18	606.6

QTL analysis

QTL mapping of trait data was carried out to examine the stages across development which in this population showed primocane fruiting habit. From the permutation test, the 95% LOD threshold in season 1 was 4.0, and in season 2 were 4.1. The QTL with LODs above these thresholds are listed in Table 3 and shown in Supplementary Figures 1-7. Several robust QTL were identified in season 1 of scoring (s1) across linkage groups 1, 2, 4, 5 and 6 and often these QTL group and co-located to a number of map regions across the linkage groups. Only one robust QTL was identified in season 2 and this is shown on LG 5 (Supplementary Figure 1).

Two QTL regions were identified in scoring season 1 from primocanes that represent the early stages of their development from closed flower buds at 59 cM, P1_s1 (21 July), to bud swell at 46 cM, P2_s1 (18 August) across LG 1 (Supplementary Figure 2). The Autumn Treasure parent was more advanced and had reached the immature fruit set stage by the 18 August; however, no bud swell had occurred by this point in Glen Fyne as expected. This region is interesting as it represents primocane bud break and was not identified in the biennial x biennial LxGM cross.

Examination of the genome region through linked markers (Supplementary Table 3) identified several interesting genes all related to early developmental processes as well as bHLH, ABA and ethylene signalling and a gene with homology to Indeterminate 1 (id1) [46] a regulator of transition to flowering possibly through modula.ing sugar transport and metabolism. Id1 acts to regulate the production of a transmissible signal in the leaf that elicits the transformation of the shoot apex to reproductive development consistent with the florigen hypothesis.

Three QTL regions were identified at 0, 17-27 and 97 cM across LG 2 (Supplementary Figure 3).

The region close to 0 cM was associated with P_PCO2_s1 and P_PCO3_s1 which represent the differential rates of ripening across genotypes. Several genes underly this region, include those expressed during flowering and flower formation such as those with a potential role in FLC regulation [47] modifications of the flower transcriptome [48] petal morphogenesis [49] as well as those involved in several other related developmental functions such as light regulation, cytochrome assembly and circadian control (Supplementary Table 3 for full list).

The region from 17-27 cM was associated with each of the primocane developmental traits recorded from July to October, representing the progression of the whole developmental process from bud swell (stage 1) to ripe fruit (stage 6) (P1_s1, P2_s1, P3_s1, P4_s1, P5_s1, P6_s1, P7_s1 and P8_s1), as well as P_PCO1_s1 representing the average rate of development across ripening. As shown in Table 3 the offspring with genotype AC have the highest mean scores and so are developing most rapidly, while AD shows intermediate values, particularly for the later scoring dates. Many interesting genes were identified here ranging from those involved in meristem identity, flowering and flower organ development, light response, and short day photoperiodism, cytochrome assembly and temperature response (Supplementary Table 3). Interestingly a gene involved in embryo development and delayed leaf senescence [50] was identified here. As days shorten and the cue is for leaf senescence to begin, delayed senescence is important for maintaining resources for later fruiting primocane types.

The region close to 97 cM was associated with P1_s1 and P_PCO2_s1 representing closed buds and early development, and again genotype AC showed faster ripening on this date. This region had few markers linking it to the raspberry genome but those scaffolds that were identified contain genes with a role in pollen and embryo development as well as a senescence regulator (Supplementary Table 3).

Three QTL regions were identified on LG4 in season one (positions 5, 76-77 and 105 cM approx.) (Supplementary Figure 4). Around 5 cM, QTLs for eight primocane traits (P2_s1 to P8_s1 and P_PCO1_s1), representing the developmental process from bud swell to ripe fruit and P_PCO1_s1 representing the average rate were detected. Genotype AC showed the fastest development, with genotype AD intermediate and BC and BD the slowest Table 3. Genes identified included those involved in light and cold regulation, plant and organ development and auxin responsive cell expansion and regulated morphogenesis (supplementary table 3).

At 77 cM two further QTLs for primocane traits were identified (P2_s1, 18 August and P3_s1, 25 August) representing stages from first bud swell to fruit set. Again, genotype AC showed the most rapid development. Here several genes with roles in developmental regulation and organ development, dormancy and flowering time related growth, fertility, flower transcriptome modification and chlorophyll biosynthesis were identified (Supplementary table 3).

At 105 cM a further region was identified for P1_s1: for this, genotype BD showed the most rapid development. Again, here few markers were available to accurately link to the genome sequence, but genes involved in embryo development and regulation of senescence were identified.

Two QTL regions were identified at 63 cM and 100 cM on across LG 5 (Supplementary Figure 1).

At 63 cM a primocane trait P_PCO2_s was identified, and the AD genotype shows the most rapid development, with slower development for BC and BD. Many genes involved in pollen germination and tube growth were identified.

At 100 cM P1_s1 (21 July) was located representing bud break: here genotype BD showed the most rapid development. This region is at the end of the LG and only one scaffold was mapped related to the genome sequence and here an expansin gene was identified that may have a role in bud swell.

One QTL region was identified at 50-57 cM across LG 6 (Supplementary Figure 5) for several primocane (P_PCO1_s1 as well as P3_s1, P4_s1, P5_s1, P6_s1 and P7_s1) (from 25 August to 8 October representing stages from bud break to Green/red fruit). The most notable feature is that genotype ab is ripening significantly faster than the other genotypes (Supplementary Figure 6) `

Genes identified here include those involved in flowering time, flower development, floral pathway promotion, early flowering and pollen germination and tube growth.

Comparisons with the previously published ripening data from the LxGM population were possible due to common markers from genome scaffolds and other previously mapped markers that could align the groups. Interestingly, in this study no overlapping developmental QTL were identified with any of the previously identified LxGM QTL from any of the traits from bud swell to ripe fruit which may suggest fruit development in these different types are under different genetic control possibly based around the vernalization requirements and the role of FLC. For a comparison of identified QTL in LxGM see gene lists in [19]. An example of the difference in regulation is particularly interesting for LG 3 (Supplementary Figure 7) which in LxGM cross has a strong effect across ripening with multiple QTL and key regulatory

genes here (FT, transport of FT (FTIP1), a gene regulating levels of FLC (EFL7) and CONSTANS-LIKE 9 (COL9) regulating CO, FT, SOC1 and SPT16 which binds to the FLC promoter and a bZIP) all with well characterized functions in photoperiod and vernalization pathways regulating biennial fruit development but has no QTL in ATxGF.

In a previous study on a tetraploid blackberry cross [25] between primocane and main season parents, the progeny in this case segregated 154:34 main season: primocane fruiting. Here [25] a locus determining primocane/floricane was identified on LG 7 which relates to LG 1 ATxGF in this study. Unfortunately, there are no common markers to determine if the location is close to QTL in this study controlling primocane fruiting trait development. In the ATxGF population the primocane fruiting is dominant unlike that in the Castro study [25]. In another raspberry study as part of an MSc programme (JA Spencer, Molecular marker analysis of primocane fruiting traits in raspberry. MSc Thesis North Carolina State University 2012, Raleigh) primocane fruiting was located on LG7, which corresponds to ATxGF LG 7 (and LG 4 of the Castro study) [25]. Another study where the primocane fruiting trait segregated identified primocane fruiting on LGs 3 and 4 which relate to ATxGF groups 2 and 7 [51].

The range of different QTL and segregation patterns across studies suggest different loci are responsible for development in the different fruiting types in raspberry and therefore different markers will be important in breeding. Future work crossing one of the ATxGF progeny with the GF parent would allow mapping of the primocane locus in this cross for comparison.

The availability of genome sequences linked to markers in this work will allow us to explore the mechanisms involved in the control of ripening processes particularly focusing on the differences between fruiting types.

CONCLUSION

This work set out to understand whether biennial or primocane fruiting was dominant in the Autumn Treasure x Glen Fyne population and to identify ripening related QTL in this population and determine how they varied from those in aLxGM (floricane season x floricane season cross). This work indicated that in this cross primocane fruiting was dominant and compatible with either the hypothesis that the primocane fruiting may be controlled by a single genetic locus or representing the chill requirement in this population being low for dormancy break, thus effectively behaving as temperature neutral in fruit development. We have identified several developmental QTL for this fruiting type across LGs 1, 2, 4, 5 and 6. These are all different from those found in the LxGM population that behaves as a main season/biennial fruiter. The work was able to examine some of the gene content underlying the QTL which further illustrated the differences in those identified in LxGM suggesting different mechanisms are involved in the regulation of development in these two populations. Again, of interest is that alleles from both parents play a role here and not just those from the primocane parent suggesting breeding for climate neutrality may be a feasible option. In terms of marker assisted selection however, it appears that different markers may be required depending on the type of crosses being generated, and other crosses need to be examined and compared using common markers linked to genome sequences to fully understand regulation and then breed for appropriate climatic factors.

REFERENCES

1. Graham J, Jennings N. Rubus spp Cane Fruit. In Biotechnology of fruit and nut crops, (2nd Edition) 2020: 606-620.
2. Fitter AH, Fitter RSR. Rapid changes in flowering time in British plants. Science 2002; 296 (5573):1689-1691.
3. Amano T, Smithers RJ, Sparke TH, Sutherland WJ. A 250-year index of first flowering dates and its response to temperature changes. Proc Royal Soc B-Biol Sci 2010; 277 (1693):2451-2457.
4. Keep E. Primocane (autumn)-Fruiting: A review with particular reference to Progress in Breeding. J Hort Sci. 1988; 63(1):1-18.
5. Jennings DL. Raspberries and Blackberries: Their Breeding, D iseases and Growth Academic Press, London. 1988.
6. Sonsteby A, Heide OM. Contrasting environmental flowering responses in annual and biennial raspberries – a mini review. 28th International Congress on science and horticulture for people/Int symposium on berries: From genomics to sustainable production, quality and health 2010; 926:221-228.
7. Kurokura T, Mimida N, Battey NH, Hytonen T. The regulation of seasonal flowering in the Rosaceae. J Exp Bot. 2013; 64(14):4131-4141.
8. Graham J, Simpson C. Developmental transitions to fruiting in red raspberry. A Study of A Primocane X Biennial Raspberry Population. 2018; 14(1):199-212.
9. Liu L, Zhang Y, Yu H. Florigen trafficking integrates photoperiod and temperature signals in Arabidopsis J Int Plant Biol 2020; 62(9):1385-1398.
10. Dodd AN, Jakobsen MK, Baker A, Telzerow A, Hou SW, Laplaze L, et al. Time of day modulates low temperature Ca2+ signals in Arabidopsis. Plant Sci.2006; 48:962-973. .
11. Yakir E, Hilman D, Hassidim M, Green R. Circadian Clock Associated 1 transcript stability and the entrainment of the circadian clock in Arabidopsis. Plant Phys. 2007; 145(3):925-932.
12. Brachi B, Faure N, Horton M, Flahauw E, Vazquez A, Nordborg M, et al. Linkage and association mapping of Arabidopsis thaliana flowering time in nature. PLOS Genetics 2010; 6(5):940.
13. Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, et al. bZIP protein mediating signals from the

floral pathway integrator FT at the shoot apex. Science. 2005; 309(5737):1052-1056.

14. Michaels S, Amasino RM. Flowering Locus C encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 1999; 11(5):949-956.
15. Michaels S, Amasino RM. Loss of Flowering Locus C activity eliminates the late-flowering phenotype of FRIGIDA and autonomous pathway mutations but not responsiveness to vernalization Plant Cell. 2001; 13(4):935-942.
16. Helliwell CA, Wood CC, Robertson M, Peacock WJ, Dennis ES. The Arabidopsis FLC protein interacts directly in vivo with SOC1 and FT chromatin and is part of a high molecular weight protein complex. Plant Journal. 2006; 42(2):183-192.
17. Searle I, He YH, Turck F, Vincent C, Fornara F, Krober S, et al. The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. Genes & Dev. 2006; 20(7):898-912.
18. Graham J, Hackett CA, 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(6):1143-1155.
19. Hackett C, Milne L, Smith K, Simpson CG, Preedy K, Graham J, et al. Enhancement of Glen Moy x Latham raspberry linkage map using GbS to further understand control of developmental processes leading to fruit ripening. BMC Genetics 2018; 19(1):59.
20. McKenzie K, Williamson S, Smith K, Woodhead M, McCallum S, Graham J. Characterisation of the Gene H region in red raspberry: Exploring its role in cane morphology, disease resistance, and timing of fruit ripening. J of Hort. 2015; 2:3.
21. Seo E, Yu J, Ryu KH, Lee MM, Lee I. WEREWOLF, a regulator of root hair pattern formation, controls flowering time through the regulation of FT mRNA stability. Plant Phys. 2011; 156(4):1867-1877.
22. Espley RV, Hellens RP, Putterill J, Stevenson DE, Kutty-Amma S, Allan AC. Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10 Plant J. 2007; 49(3):414–427.
23. Allan AC, Hellens RP, Laing WA. MYB transcription factors that colour fruit. Trends in Pl Sci. 2008; 13(3):99-102.
24. Jin HL, Martin C. Multifunctionality and diversity within the plant MYB-gene family Plant Mol Biol. 1999; 41:577-585.
25. Castro P, Stafne ET, Clark JR, Lewers KS. Genetic mapping of the primocane fruiting and thornless traits of tetraploid blackberry Theor Appl Genet. 2014; 126(10): 2521-2532.
26. Graham J, Marshall B, Squire GR. Genetic differentiation over a spatial environmental gradient in wild Rubus idaeus populations. New Phyt. 2003; 157:667-675.
27. Graham J, Smith K, MacKenzie K, Jorgensen L, Hackett CA, 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.
28. Stafne ET, Clark JR, Weber CA, Graham J, Lewers KS. Simple sequence repeat (SSR) markers for genetic mapping of raspberry and blackberry J Am Soc Hort Sci. 2005; 130(5):722-728. [Cross Ref] [Research Gate]
29. Woodhead M, McCallum S, Smith K, Cardle L, Mazzitelli L, Graham J. Identification, characterisation and mapping of simple sequence repeat (SSR) markers from raspberry root and bud ESTs. Mol Breed. 2008; 22:555-563.
30. Woodhead M, Weir A, Smith K, McCallum S, MacKenzie K, Graham J. Functional markers for red raspberry J Am Soc Hort Sci. 2010; 135(5):418-427. [Cross Ref] [Research Gate]
31. Kassim A, Poette J, Paterson A, Zait D, McCallum S, Woodhead M, et al. Environmental and seasonal influences on red raspberry anthocyanin antioxidant contents and identification of quantitative trait loci (QTL) Mol Nut Food Res. 2009;53:625-634.
32. McCallum S, Woodhead M, Hackett CA, Kassim A, Paterson A, Graham J. Genetic and environmental effects influencing fruit colour. Theor Appl Genet. 2010; 121:611-627.
33. Castillo NRF, Reed BM, Graham J, Fernandez F, Bassil NV. Microsatellite markers for raspberry and blackberry. J Am Soc Hort Sci. 2010;135(3):271-278.
34. Paterson A, Kassim A, McCallum S, Woodhead M, Smith K, Zait D, et al. Environmental and seasonal influences on red raspberry flavour volatiles and identification of quantitative trait loci (QTL) and candidate genes. Theor Appl Genet. 2013;126:33-48.
35. Simpson C, Cullen D, Hackett C, Hallett P, McNicol J, Graham J. Mapping and expression of genes associated with raspberry fruit ripening and softening Theor Appl Genet. 2016;130(2): 159-172.
36. Foster T, Bassil N, Dossett M, Worthington ML, Graham J. Genetic and genomic resources for Rubus breeding: a roadmap for the future. HORTRES. 2019;6:116.
37. Van Ooijen JW. Joinmap*4 Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen, Netherlands. 2006. [KIPDF]
38. Cullis BR, Smith AB, Coombes NE. On the design of early generation variety trials with correlated data J Agri Biol Env Stat. 2006;1:381–393.
39. Van Ooijen JW. MapQTL *5 Software for the mapping of quantitative trait loci in experimental populations. Kyazma BV, Wageningen, Netherlands. 2004.
40. Graham J, Smith K, Tierney I, MacKenzie K, Hackett C. Mapping gene H controlling cane pubescence in raspberry

and its association with resistance to cane botyritis and spur blight, rust and cane spot. Theor Appl Genet. 2006;112:818-831.

41. Graham J, Hackett CA, Smith K, Woodhead M, MacKenzie K, Tierney I, et al. 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.
42. Graham J, Hackett CA, Smith K, Karley A, Mitchell C, Roberts H, et al. Genetic and environmental regulation of plant architectural traits and opportunities for pest and disease control in raspberry Ann Appl Biol. 2014;165 (3):318-328.
43. Graham J, Smith K, McCallum S, Hedley P, Cullen D, Dolan A, et al. Towards an understanding of crumbly fruit in red raspberry Springer Plus 2015;4:223.
44. Woodhead M, Weir A, Smith K, McCallum S, Jennings N, Hackett CA, et al. Identification of QTLs for cane splitting in red raspberry (Rubus idaeus). Mol Breed. 2013;31:111-122.
45. Graham J, Smith K, Tierney I, MacKenzie K, Hackett C. Mapping gene H controlling cane pubescence in raspberry and its association with resistance to cane botyritis and spur blight, rust and cane spot. Theor Appl Genet. 2006;112:818-831.
46. Seo E, Yu J, Ryu KH, Lee MM, Lee I. WEREWOLF, a regulator of root hair pattern formation, controls flowering time through the regulation of FT mRNA stability. Plant phys. 2011:156:1867-1877.
47. Berr A, Xu L, Gao J, Cognat V, Steinmetz A, Dong A, et al. Set domain group 25 encodes a histone methyltransferase and is involved in FLC activation and repression of flowering. Plant phys. 2009;151(3):1476-1485.
48. Sudre D, Gutierrez-Carbonell E, Lattanzio G, Rellan-Alvarez R Gaymard F, Wohlgemuth G.et al. Iron-dependent modification of the flower transcriptome, proteome, metabolome, and hormonal content in an Arabidopsis ferritin mutant J Exp Bot. 2013;64(10):2665-2688
49. Yang Y, Chen B, Dang X, Zhu LL, Rao J, Ren HB. et al. Arabidopsis IPGA1 is a microtubule-associated protein essential for cell expansion during petal morphogenesis. J Exp Bot. 2019;70(19):5231-5243.
50. Chen C, Galon Y, Ishka MR, Malihi S, Shimanovsky V, Twito S. et al. Ascorbate peroxidase6 delays the onset of age-dependent leaf senescence. Plant Phys. 2020;185(2):441-456
51. Jibran R, Spencer J, Fernandez G, Montfort A, Mnejja M, Dzierzon H, et al. Two Loci, RiAF3 and RiAF4, Contribute to the Annual Fruiting Trait in Rubus. Frontiers in Pl Sci. 2019;1341.