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ORIGINAL ARTICLE

Quantitative trait loci (QTL) mapping of blush skin and flowering time in a European pear (*Pyrus communis*) progeny of 'Flamingo' × 'Abate Fetel'

Solomon M. Ntladi[1,2] · Jan P. Human[1] · Cecilia Bester[1] · Jessica Vervalle[2] · Rouvay Roodt-Wilding[2] · Kenneth R. Tobutt[1]

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Abstract

Blush skin and flowering time are agronomic traits of interest to the Agricultural Research Council (ARC) Infruitec-Nietvoorbij pear breeding programme. The genetic control of these traits was investigated in the pear progeny derived from 'Flamingo' (blush cultivar) × 'Abate Fetel' (slightly blush) made up of 121 seedlings. Blush skin was scored phenotypically over three seasons and flowering time was scored over two seasons. A total of 160 loci from 137 simple sequence repeat (SSR) markers were scored in the progeny and used to construct parental genetic linkage maps. Quantitative trait loci (QTL) analysis revealed two QTLs for blush skin, a major QTL on linkage group (LG) 5 in 'Flamingo', and a major QTL on LG9 in 'Abate Fetel'. Two SSR markers, NB101a and SAmsCO865954, were closely linked with the major QTL on LG5 in 'Flamingo', with alleles 139 bp and 462 bp in coupling, respectively. These markers were present in approximately 90% of the seedlings scored as good blush (class 4) based on the average data set. These two markers were used to genotype other pear accessions to validate the QTL on LG5 with the view of marker-assisted selection. Two candidate genes, *MYB86* and *UDP-glucosyl transferase*, were associated with the QTL on LG5 and *MYB21* and *MYB39* were associated with the QTL on LG9. QTL analysis for flowering time revealed a major QTL located on LG9 in both parents. Marker GD142 with allele 161 bp from 'Flamingo' was present in approximately 88% of the seedlings that flowered earlier than either parent, based on the average data set. The QTLs and linked markers will facilitate marker-assisted selection for the improvement of these complex traits.

Keywords European pear · Blush skin · Flowering time · Candidate genes · SSRs · QTLs

Introduction

The European pear (*Pyrus communis* L.) is an economically important rosaceous fruit crop of temperate regions and is grown in South Africa, with about half of the crop being exported. Traits of interest in pear breeding at the Agricultural Research Council (ARC) include blush skin colour (Human 2013) and adaptation to mild winter, e.g. early flowering time. Blush pear cultivars fetch a higher price than fully red or green pears in overseas fresh markets (Human 2013).

The blush skin colour in pear is manifested as red pigmentation, often covering more than a third of the surface of mature fruit well exposed to the sun. Traditional blush pear cultivars include 'Bella di Giugno', 'Colorée de Juillet', 'Corella', 'Forelle', 'Nordhäuser Winterforelle' and 'Seckel', all chance seedlings. Some recent blush pear cultivars released from various pear breeding programmes include 'Cheeky' (parentage not confirmed) (Human and von Mollendorff 2009) from South Africa; 'Celina' ('Coloree de Juillet' × 'Williams') from Norway (Hjeltnes 2015); and 'Deliza' ('Corella' × 'Doyenne du Comice') and 'Lanya' ('Butirra Precoce Morettini' × 'Corella') from Australia (Turpin et al. 2016). The blush skin

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✉ Rouvay Roodt-Wilding
roodt@sun.ac.za

[1] Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa

[2] Molecular Breeding and Biodiversity Group, Department of Genetics, Stellenbosch University, Private Bag XI, Stellenbosch 7602, South Africa

trait differs from the complete red skin colour trait reported in certain sports such as 'Max Red Bartlett', which has been attributed to the *C* gene (Zielinski 1963).

The reported anthocyanin compounds associated with the red skin colouration in pears include cyanidin-3-galactoside, cyanidin-3-arabinose and quercetin-3-0-glucoside (Francis 1970; Dussi et al. 1995; Pierantoni et al. 2010). Biosynthesis and degradation of these compounds are affected by environmental factors such as light and temperature (Dussi et al. 1995; Steyn et al. 2004a, b). Under South African conditions, high temperature and high irradiance promote the degradation of anthocyanin, which reduces the blush colour in cultivars such as 'Rosemarie' (Steyn et al. 2004a, b).

Anthocyanin accumulation is controlled by structural and regulatory genes (Xie et al. 2011; Yang et al. 2015a). Structural genes encode enzymes involved in anthocyanin biosynthesis pathways such as phenylalanine ammonia-lyase (PAL); chalcone isomerase (CHI); chalcone synthase (CHS); flavanone 3-hydroxylase (F3H); anthocyanidin synthase (ANAS); dihydroflavonol 4-reductase (DFR); and UDP-glucose: flavonoid 3-O-glucosyl transferase (UFGT). Regulatory genes encode transcription factors that regulate expression of the structural genes and include the MYB transcription factor, a basic helix-loop-helix transcription factor and WD40-repeat proteins (Baudry et al. 2004; Xie et al. 2011; Li et al. 2012; Yang et al. 2015b) which interact and form transcription complexes regulating anthocyanin biosynthesis (Feng et al. 2010).

Genetic studies on blush skin colour in the European pear have been reported only by Zielinski et al. (1965) and Human (2013). Zielinski et al. (1965) found that three progenies derived from 'Doyenne du Comice' (non-blushed skin) crossed with three different seedlings of the cross 'Seckel' (blushed skin) × 'Farmingdale' (non-blushed skin) segregated in a ratio of 1:1 of blushed versus non-blushed and proposed that blush skin colour was controlled by a single dominant allele inherited from 'Seckel'. Human (2013) observed a slight deviation to the 1:1 ratio in a progeny derived from 'Flamingo' (blush) × 'Abate Fetel' (green to slight blush depending on the season, and russetted) and in some related progenies. Human proposed a complimentary epistasis model where blush is controlled by two recessive genes with parental genotypes *bbCc* (blush) and *BbCc* (green) resulting in a 5:3 ratio. In the Asian pear, Volz et al. (2008) speculated that two complementary dominant genes control blush skin colour in the cultivar 'Huobali'.

In other rosaceous fruit species such as sweet cherry (*Prunus avium* L.), peach (*Prunus persica* (L.) Batsch) and apple (*Malus pumila* Mill.), mapping studies have mapped red skin colouration as a monogenic trait (Maliepaard et al. 1998; Yamamoto et al. 2001) and blush skin as a polygenic trait (Sooriyapathirana et al. 2010; Frett et al. 2014). In sweet cherry, a major quantitative trait loci (QTL) located on linkage group (LG) 3 and minor QTLs on LG6 and LG8 for red skin colouration were reported (Sooriyapathirana et al. 2010). The *PavMYB10* regulatory gene, suggested as determining skin colour, was found to co-locate within the major QTL on LG3. In peach, a single dominant gene, *Sc*, associated with red skin pigmentation was mapped to LG3 (Yamamoto et al. 2001). A further study in peach reported a QTL for blush skin on LG4 (Cantín et al. 2010) and subsequently Frett et al. (2014) found four QTLs associated with blush skin: a major QTL located on LG3, two minor QTLs on LG4 and a minor QTL on LG7. The major QTL on LG3 co-located with the *PprMYB10* gene. One of the minor QTLs on LG4 co-located with the structural gene uridine diphosphate (UDP)-glycosyl transferase. In apple, a single dominant gene, *Rf*, responsible for red skin colouration was mapped to LG9 (Maliepaard et al. 1998). Various MYB transcription factors, including orthologous *MYB10* genes, have been reported to be responsible for red and blush skin colouration in apple including *MdMYB1* (Takos et al. 2006), *MdMYBA* (Ban et al. 2007) and *MdMYB10* (Espley et al. 2007). A recent study by Chagné et al. (2016) mapped and confirmed locus *MYB10*, located on LG9, to be responsible for red skin colouration in apple. Pierantoni et al. (2010) mapped the pear orthologue, *PcMYB10*, on LG9 in 'Max Red Bartlett' × 'Abate Fetel' and found elevated expression of *PcMYB10* in the red bud mutation 'Max Red Bartlett' compared to the original yellow cultivar 'Williams'. In Asian pear, the *PcMYB10* gene was reported to be associated with the red colouration of young leaves, fruit skin and flower buds (Feng et al. 2010). A recent study by Yao et al. (2017) reported the *PyMYB114* gene located on LG5 to be associated with red skin colouration in the pear 'Bayuehong'. Yang et al. (2015b) investigated the expression levels of three transcription factors, *PcMYB10*, *PcbHLH33* and *PcWD40*, in occidental red pears, where skin colour is more intense at fruit set and decreases during fruit maturity. The expression of *PcMYB10* and *PcbHLH33* corresponded with the anthocyanin variation found in these pears, with elevated expression during the early stages and decreasing expression during the ripening process. On the contrary, *PcWD40* showed an inverse relationship, reaching maximum expression at the mature stage, when anthocyanin content is at its lowest, suggesting that these transcription factors are involved in the regulation of anthocyanin synthesis. Although these transcription factors are associated with anthocyanin regulation, the mapped position of *PcMYB10* does not coincide with the red colour locus that was mapped to LG4 by Dondini et al. (2008), suggesting that there are other genes also contributing to this trait. Additional genes in the *MADS* (*PyMADS18*), *AP2* and *WRKY* (*WARK*) transcription factor families have also been found to be differentially expressed between red and green pears in European pear (Wu et al. 2013; Yang et al. 2015a). These genes have been identified and investigated in red skinned pears, and no *MYB* genes have been associated with the blush trait as of yet.

Flowering time is a phenological trait of horticultural economic interest with respect to the adaptation of cultivars both to warm and frosty areas. This trait is regulated both by genetic and environmental factors (Jung and Müller 2009). Environmental factors such as winter chilling contribute to release of endodormancy, allowing budbreak, and heat accumulation during spring promotes the growth of floral buds. Genetic control of flowering time in pear is still poorly understood and there is little literature on this subject. In apple, many genetic factors may be involved. A study by Hauagge and Cummins (1991) reported that the exceptionally early flowering of the cultivar 'Anna' is controlled by at least a major dominant gene and other studies have found QTLs located on LG7, LG10 and LG17 (Liebhard et al. 2003) and LG8 and LG15 (Kunihisa et al. 2014) that control flowering time. QTLs associated with floral budbreak in apple were reported on LG1, LG6, LG8, LG9, LG12 and LG17 (Celton et al. 2011). Recently, QTLs associated with flowering time in five different apple progenies were reported on LG7, LG8, LG9, LG12 and LG15 (Allard et al. 2016). The same study found candidate genes, *agamous-like 24* (*AGL24*) and *flowering locus C-like gene* (*FLC-like*), associated with flowering time located on LG9. In European pear, a recent study by Gabay et al. (2017) reported QTLs associated with timing of vegetative budbreak in the progeny of 'Spadona' (low chill) × 'Harrow Sweet' (high chill), and found three QTLs including a major and minor QTL on LG9 and a minor QTL on LG8.

Several genetic linkage maps have become available for the European pear in recent years (Yamamoto et al. 2002c; Dondini et al. 2004; Yamamoto et al. 2004; Pierantoni et al. 2007; Yamamoto et al. 2007; Dondini et al. 2008; Nishitani et al. 2009; Bouvier et al. 2012; Yamamoto et al. 2013; Montanari et al. 2013; Knäbel et al. 2015). These maps, covering all 17 LGs, were constructed mostly using simple sequence repeat (SSR) markers derived from apple and pear and, recently, single-nucleotide polymorphisms (SNPs) derived from apple and pear. Various major genes have been mapped, including the *S*-locus for self-incompatibility (Yamamoto et al. 2004), *C* for red skin colour (Dondini et al. 2008), *PcDw* for dwarf habit (Wang et al. 2011) and *Rvp1* for resistance to pear scab *Venturia pyrina* (Bouvier et al. 2012), as well as QTLs for polygenic traits such as resistance to fire blight (Le Roux et al. 2012; Montanari et al. 2016) and date of vegetative budbreak (Gabay et al. 2017).

As traditional breeding is a lengthy process, the pear breeding programme at the ARC is adopting molecular markers, firstly to map traits of interest and secondly to implement marker-assisted selection (MAS). In the present study, the genetic control of blush skin and flowering time in a progeny derived from 'Flamingo' × 'Abate Fetel' and the mapping with SSRs of QTLs associated with these traits are reported. In addition, validation of marker alleles associated with these traits in other pear accessions is presented.

Materials and methods

Plant material

A progeny comprising of 121 seedlings derived from the cross between 'Flamingo' × 'Abate Fetel' was used. 'Flamingo' is a blushed cultivar with about 50% blush and medium flowering time, derived from the cross 'Bon Rouge' (complete red sport of 'William's Bon Chretien') × 'Forelle' (blushed). 'Abate Fetel' is a chance seedling with a slight blush of about 20–50% depending on the season, but the skin is predominantly russetted and this cultivar has a medium flowering time. Parents were planted at Elgin Research Farm (34.082098° S, 19.011826° E) and Bien Donné Research Farm (33.8392° S, 18.9726° E). Seedlings were planted at Drostersnes Research Farm (34.04314° S, 19.04365° E), approximately 8.5 km from the Elgin Research Farm. Seedlings were planted between 2002 and 2004 on their own roots and at a spacing of 1 m within the rows and 4.5 m between the rows. Standard orchard management practices such as fertilisation, irrigation and spraying fungicides and pesticides were applied to the progeny but the trees were not pruned. To investigate the possible effect of weather on the blush skin trait and flowering time, weather data was collected from the Riviera weather station (34.056233° S, 19.132233° E) that is located 4.35 km from the Drostersnes Research Farm. Parameters recorded for blush skin were daily minimum and daily maximum temperatures, whereas for flowering time, the recorded parameters were accumulated cold and heat units.

Methods

Phenotyping of blush skin and flowering time

Skin colour was scored towards the end of the pear ripening season of 2013, 2014 and 2015 in the Drostersnes orchard for the seedlings and in the Elgin Research Farm for the parents. During 2015, parents were also scored at the Bien Donné Research Farm. A visual quantitative scale ranging from 0 (no blush) to 5 (excellent blush) (Fig. 1) was used. During 2013, the seedlings were scored on two different dates (14 and 25 February). During the 2014 season, seedlings were scored on four different dates (13, 22, 29 January and 6 February). During the 2015 season, in which the pears ripened earlier, seedlings were scored on three occasions (2, 15 and 21 January). On each occasion, the trait was scored under sunny conditions and only from the side of the tree facing the sun where maximum expression of colour is expected. The scores based on associated percentage assigned to each seedling (typically 10 or more fruits) were used for normality distribution tests and QTL analysis.

The flowering time was recorded during the spring of 2014 and 2015 by visually estimating the percentage of open flowers on the spur shoots of the whole tree. During the spring of 2014, flowering was scored once a week (every 5–6 days) over a period of 6 weeks.

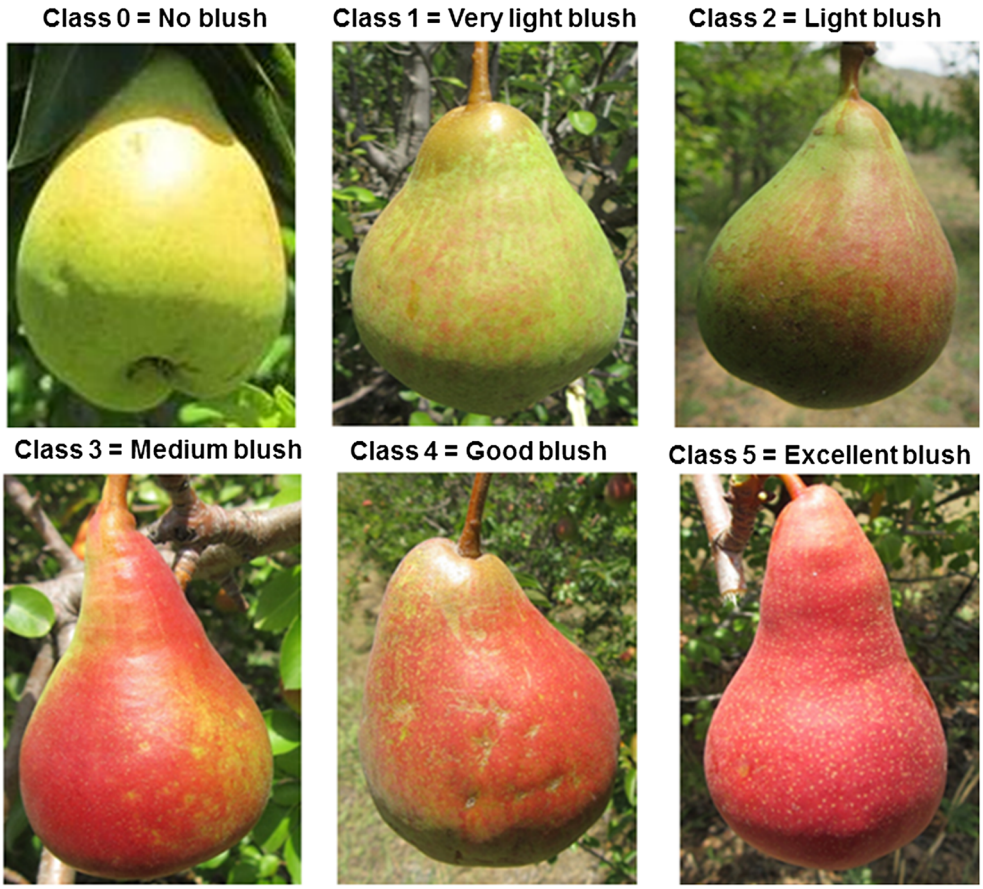


Fig. 1 Six different classes of blush skin of pear fruit scored visually in the orchard from the progeny derived from 'Flamingo' × 'Abate Fetel'

During 2015, flowering was scored twice a week (every 2–3 days) between September and October over a period of 6 weeks. The Julian date (the number of days from 1 January) at which 80% of the flowers were open was used for QTL analysis.

Statistical analysis of the traits

The repeatability or intra-class correlation coefficient (t) to determine the resemblance between related individuals was calculated as $t = \sigma^2_b/(\sigma^2_b + \sigma^2_w)$ according to the formula described (Falconer and Mackay 1996), where σ^2_b represents the variance between the trees within the progeny and ($\sigma^2_b + \sigma^2_w$) represents the total phenotypic variance within the progeny, including year × tree interaction. The phenotypic data were subjected to statistical analysis in XLSTAT, according to the method used by Kunihisa et al. (2014). Normality of the distribution of the data was tested using a Kolmogorov-Smirnov test at $P < 0.05$ and kurtosis and skewness tests. Histograms were drawn for each trait to allow for comparison between seedlings and parents. The phenotypic values of each seedling scored for each trait were subjected to an analysis of variance (ANOVA) in a one-way classification, using seedling as a factor. The statistical model used to calculate the ANOVA was expressed as:

$$p_{ij} = \mu + g_i + e_{ij}\ (i = 1, 2, \ldots, G;\ \ j = 1, 2, \ldots, Ni),$$

where p_{ij} represents the phenotypic value of the seedlings for the ith seedling in the jth replicate, μ represents the grand mean, g_i represent a genotypic value of the ith seedling and e_{ij} represents the residual following $N(0, \sigma^2_e)$, where G represents the number of seedlings scored for the trait and Ni represents the number of replicates for each seedling. The number of replicates was represented by the number of years each seedling was scored. The statistical analysis was performed by using PROC GLM within the software SAS© (SAS Institute Inc.).

Inheritance of blush skin was tested for a segregation ratio of 5:3 proposed by Human (2013) using a chi-squared test ($\chi 2$) in all 3 years and for the average data set. Seedlings in class 0 (no blush) were contrasted with the seedlings in classes 1 to 5 (very light blush to excellent blush) grouped together as 'blushed' in accord with the grouping used by Human (2013). Additionally, seedlings in classes 0 and 1 were contrasted with the seedlings in classes 2 to 5 and seedlings in classes 0, 1 and 2 were contrasted with the seedlings in classes 3 to 5.

DNA extraction

Young leaf samples were collected from the parents and seedlings during the spring of 2012. DNA was extracted using a modification of the CTAB method by Doyle and Doyle (1990)

with the addition of 10% polyvinylpyrrolidone to remove secondary plant products such as polyphenolics.

SSR markers and PCR amplification

A subset of 189 SSR markers distributed across all 17 LGs at approximately 15–20-cM intervals were selected from different reference maps and tested for polymorphism in the two parents, 'Abate Fetel' and 'Flamingo', and subsets of six seedlings (Table 1). Progeny that did not display trueness to parentage was excluded from further analysis. The forward primers were labelled with a fluorescent dye. Some primer pairs were multiplexed (Table S1, Supplementary Material) and other primer pairs were singleplex. Each PCR reaction comprised the following: a 10 μl PCR mixture containing 5 μl Qiagen Multiplex PCR kit (Qiagen), 0.2 μM of each primer, 2 μl H_2O and 20–30 ng/μl DNA template. The PCR amplifications were performed using a G-Storm Thermal Cycler (Vacutec, South Africa) using the 'Type it' microsatellite kit (Qiagen) according to the manufacturer's instruction as follows: an initial denaturation step of 95 °C for 15 min was followed by 25 cycles of 94 °C for 30 s, an annealing temperature of 60–50 °C for 90 s decreasing by 1 °C for the first 10 cycles, then 72 °C for 60 s followed by a final extension step of 30 min at 60 °C. Reaction products were sized at Stellenbosch University Central Analytical Facility (CAF) on an ABI 3130xl or ABI 3730xl DNA sequencer (Applied Biosystems) using GeneScan 500 (-250) LIZ™ as an internal size standard. The allele sizes scored were verified independently by a co-worker.

Locus segregation and construction of genetic linkage maps

Chi-squared tests (χ^2) of goodness of fit to expected segregation ratios of markers of 1:1:1:1, 1:1 and 1:2:1 were calculated using JOINMAP4.1 (Van Ooijen 2006). However, markers with null alleles and expected segregation ratios of 1:1:2 and 2:1:1 were included in linkage analysis and coded as nnxnp and lmxll, respectively. For SSR markers that detected multiple loci, the loci were distinguished by suffixes assigned by previous authors or by adding new suffixes such as *w*, *x*, *y* or *z* to the SSR marker name. Markers with less than 2% missing data were used for the construction of the linkage maps. Loci were grouped using a logarithm of odds (LOD) score of 3.0. The regression mapping algorithm option of JOINMAP4.1 (Van Ooijen 2006) was used to construct the linkage maps. Map distances were calculated using Kosambi's map function (Kosambi 1944) with a maximum recombination frequency of 0.40. Linkage group numbers and marker orders were established by comparison with the published pear maps

Table 1 Sets of SSR primers developed in apple or pear from which primers were chosen for segregation analysis in the pear progeny of 'Flamingo' × 'Abate Fetel' on the basis of their published map positions. The reference is to the original paper that developed the marker and not to the publication in which it was mapped

Primer set	Type	Number tested	Reference
Malus pumila			
CH, MS	Genomic	36	Liebhard et al. 2002
CN, Hi	Genomic	16	Silfverberg-Dilworth et al. 2006
CN, CT, CG, CO	EST	13	Gasic et al. 2009; Han et al. 2011
GD	Genomic	6	Hokanson et al. 1998; Hemmat et al. 2003
MEST	Genomic	5	Moriya et al. 2012
NZ	Genomic	1	Guilford et al. 1997
NZms	Genomic	7	Celton et al. 2009
SAms	EST	16	Van Dyk et al. 2010
Pyrus communis			
EMPc	Genomic	9	Fernández-Fernández et al. 2006
Pyrus pyrifolia			
BG, K	Genomic	5	Yamamoto et al. 2002a
IP	Genomic	4	Inoue et al. 2007
Nau	Genomic	12	Chen et al. 2015
NB	Genomic	16	Yamamoto et al. 2002c
NH	Genomic	23	Yamamoto et al. 2002b, 2007
RL	Genomic	1	Yamamoto et al. 2002c
TsuE	EST	16	Nishitani et al. 2009
TsuG	EST	3	Yamamoto et al. 2013
Total		189	

(Yamamoto et al. 2013; Chen et al. 2015) and apple maps (Fernández-Fernández et al. 2008; Van Dyk et al. 2010; Han et al. 2011; Fernández-Fernández et al. 2012; Moriya et al. 2012). Map figures were created using MAPCHART2.2 (Voorrips 2002).

QTL analyses for mapping traits

For QTL analysis of blush skin, scores of 103 seedlings in 2013, 116 seedlings in 2014 and 119 seedlings in 2015 were analysed individually and as an average data set of all 121 seedlings (Table S2, Supplementary Material). QTL analysis was also performed on groupings of blush data (blush vs no blush) based on different class groupings (0, 1 vs 2, 3, 4, 5 and 0, 1, 2 vs 3, 4, 5). For QTL analysis of flowering time, scores of 115 seedlings in 2014 and 114 seedlings in 2015 were analysed individually and as an average data set of 108 seedlings (Table S3, Supplementary Material). Seedlings for which data were only available for 1 year were excluded from the the average data set and subsequent QTL analysis.

Detection of QTLs for the traits was done using MAPQTL6.0 (Van Ooijen 2009). QTL data for blush skin and flowering time, which displayed a normal phenotypic distribution, were initially analysed using the Kruskal-Wallis test (K-W). For the second step, interval mapping (IM) was performed to identify markers with the highest LOD associated with the blush skin as the cofactors. Only markers with the highest LOD scores and percentage variance were used as cofactors in the restricted Multiple QTL Method (rMQM). Following rMQM, a permutation test with 1000 replications of the phenotypic data was performed to identify the genome-wide significant LOD score of $P < 0.05$. QTLs detected with LOD scores higher than the genome-wide threshold were considered significant. Moreover, QTLs detected with rMQM in more than 1 year and based on the average data sets were considered stable QTLs. The QTL positions detected on different LGs are presented by drawn bar segments using MAPCHART2.2 (Voorrips 2002). The maximum LOD score of each QTL was generated in MAPQTL6.0. The QTL confidence intervals were set as the map interval corresponding to a decline of 1 and 2 LOD units on either side of the maximum LOD score using MAPCHART2.2.

Statistical analysis of the SSR marker alleles associated with QTLs for blush skin on LG5 and LG9 and flowering time on LG9 was conducted by assessing type I errors (α) and type II errors (β) (Hühn and Piepho 2003; Ramkumar et al. 2011). The power ($1-\beta$) test for the linkage of the marker with both traits was also conducted (Hühn and Piepho 2003). The sensitivity (which is the probability that the test will be positive if a seedling has inadequate blush or early flowering) and specificity (which is the probability that the test will be negative if the seedling does not have an inadequate blush skin or have late flowering period) were also calculated.

Candidate gene identification for blush skin in pear

The annotated apple genome was used to identify candidate genes associated with anthocyanin biosynthesis in the QTL region for blush skin on LG 5 and LG9, due to partial anchoring of the European pear genome, preventing analysis for the location of candidate genes (Velasco et al. 2010). Apple SSR markers flanking the identified QTL intervals were obtained from the reference map reported by Fernández-Fernández et al. (2012). On LG5, the genomic region between SSR markers CH03a09 (16.3 cM) and NZmsEB132749 (29.6 cM) was investigated for potential anthocyanin genes, whereas on LG9, the genomic region between SSR markers NZmsCN943946 (0.0 cM) and NZmsEB116209 (23.9 cM) was investigated. The identified apple candidate genes were subsequently used to identify the syntenous pear gene models through reciprocal BLAST search.

Validation of marker alleles associated with blush skin in other blush pear cultivars and selections

The two SSR markers closest to the QTL region on LG5, NB101a and SAmsCO865954, were screened in 25 pear accessions (including 16 accessions derived from 'Forelle') made up of cultivars, sports and selections (Table 2) to evaluate the potential of these markers as tools for MAS for breeding of blush cultivars.

Results

Inheritance of blush skin

The scores in the 121 seedlings of the 'Flamingo' × 'Abate Fetel' progeny over the years 2013, 2014 and 2015 ranged from 0 to 5. 'Flamingo' displayed a constant score of 3 throughout, while 'Abate Fetel' varied between the years from 0 to 3. Some of the seedlings scored higher, 4 or 5, in the 2013 and 2015 seasons than the parents, in comparison to the 2014 season, indicative of transgressive segregation. However, the majority of seedlings scored 2 or 3 in all years. The distribution for blush skin in the progeny is shown in Fig. 2. According to the Kolmogorov-Smirnov test, the 2013 data set was normally distributed with $P = 0.042$ (Fig. 2a). The 2014 (Fig. 2b), 2015 (Fig. 2c) and average (Fig. 2d) data sets deviated from normality. However, the kurtosis test of the 2014 data gave an absolute value of 0.311 larger than the negative skewness value of − 0.370, suggesting the data were normally distributed. Similarly, for 2015, the kurtosis test gave a positive value of 0.129, larger than the skewness with a negative value of − 0.040, indicative of normal distribution. The average data set showed an absolute kurtosis value of 0.772 larger than the negative skewness value of − 0.250, indicative of normal distribution. Due to the normal

Table 2 The 25 pear accessions used to validate two of the markers associated with blush skin. Allele sizes of the markers NB101a and SAmsCO865954 associated with blush are indicated in italics

Accessions	Parentage	Skin colour scores	NB101a (bp)	SAmsCO865954 (bp)
Bon Rouge	Sport of William's Bon Chretien	6	163/178	430/456
Carmen	Dr. Jules Guyot × Bella di Giugno	2	146/163	456/460
Celina	Coloree de Juillet × Williams	3	146/155	460/*462*
Coscia	Chance seedling	1	153/163	456/456
Flamingo	Bon Rouge × Forelle	3	*139*/178	430/456/*462*
Forelle	Chance seedling	3	*139*/163	456/*462*
Gerburg	Clapps Favourite × Nordhäuser Winterforelle	3	163/178	456/456
Gräfin Gepa	Nordhäuser Winterforelle × Baierschmidt	4	163/163	456/456
Hortensia	Nordhäuser Winterforelle × Clapps Favourite	3	155/163	456/*462*
P07-3	Flamingo × Harrow Delight	3	*137*/155	456/*462*
Rosemarie	Bon Rouge × Forelle	3	*139*/163	456/*462*
Thimo	Nordhäuser Winterforelle × Madame Verté	3	163/178	456/456
5-40-45	Bon Rouge × Forelle	4	163/178	430/456
5-40-60	Bon Rouge × Forelle	3	163/178	427/452
5-41-18	Bon Rouge × Forelle	3	163/178	430/456
8-23-81	Bon Rouge × Forelle	4	*137*/163	430/456/*462*
8-24-51	Bon Rouge × Forelle	2	*139*/178	430/456/*462*
8-25-25	Bon Rouge × Forelle	2	163/163	456/456
8-25-57	Bon Rouge × Forelle	1	163/163	456/456
8-25-72	Bon Rouge × Forelle	2	*139*/178	430/456/*462*
8-30-145	Bon Rouge × Forelle	1	*137*/163/178	430/456/*462*
8-31-158	Bon Rouge × Forelle	3	*139*/178	430/456/*462*
8-31-23	Bon Rouge × Forelle	0	*137*/178	456/*462*
8-31-67	Bon Rouge × Forelle	2	*139*/178	430/456/*462*
8-33-53	Bon Rouge × Forelle	1	*139*/178	430/456/462

0 = no blush, 1 = very light blush, 2 = light blush, 3 = medium blush, 4 = good blush, 5 = excellent blush, 6 = purple red

distribution of the data for these years, the data sets were suitable for QTL analysis. The repeatability for blush skin was calculated to be 0.47 based on all genotypes scored over all 3 years, which suggests that scoring of the trait was inconsistent over the years.

Comparison of blush development across all 3 years showed a greater expression of the trait during 2015 than during the 2013 and 2014 seasons in both progenies, indicating the effect of the environmental conditions such as temperature on blush development. Figure 3 shows the average weekly minimum and maximum temperature recorded during developmental stage from beginning of December to commercial harvesting during January and February. In comparison, the growing season of 2015 was very cold and short with the average maximum temperature of 25 °C, which appeared to promote accumulation of the anthocyanin, resulting in better blush development compared to the warm season of 2013 and 2014 with an accumulated average temperature of 27 °C. The high average maximum temperature accumulated during the 2013 and 2014 seasons could have contributed to the degradation of anthocyanin accumulation and loss of red skin colour in these progenies.

According to Human (2013), the parental genotypes of 'Flamingo' (*bbCc*, blush skin) and 'Abate Fetel' (*BbCc*, green skin) gave the expected segregation ratio of 5:3 if genotypes *bbCc*, *bbCC*, *bbCc*, *bbcc* and *Bbcc* are grouped as blush with *BbCC* and *BbCc* grouped as green. A χ^2 test of blush (classes 1 to 5) versus non-blush (class 0) revealed that none of the data sets were consistent with the expected segregation ratio of 5:3. A χ^2 test of blush (classes 2 to 5) versus non-blush (classes 0 to 1) revealed that only the 2013 data was consistent with the expected segregation ratio of 5:3. A χ^2 test of blush (classes 3 to 5) versus non-blush (classes 0 to 2) revealed that the 2015 and average data sets were consistent with the expected segregation ratio of 5:3 but segregation of the 2013 and 2014 data sets was not.

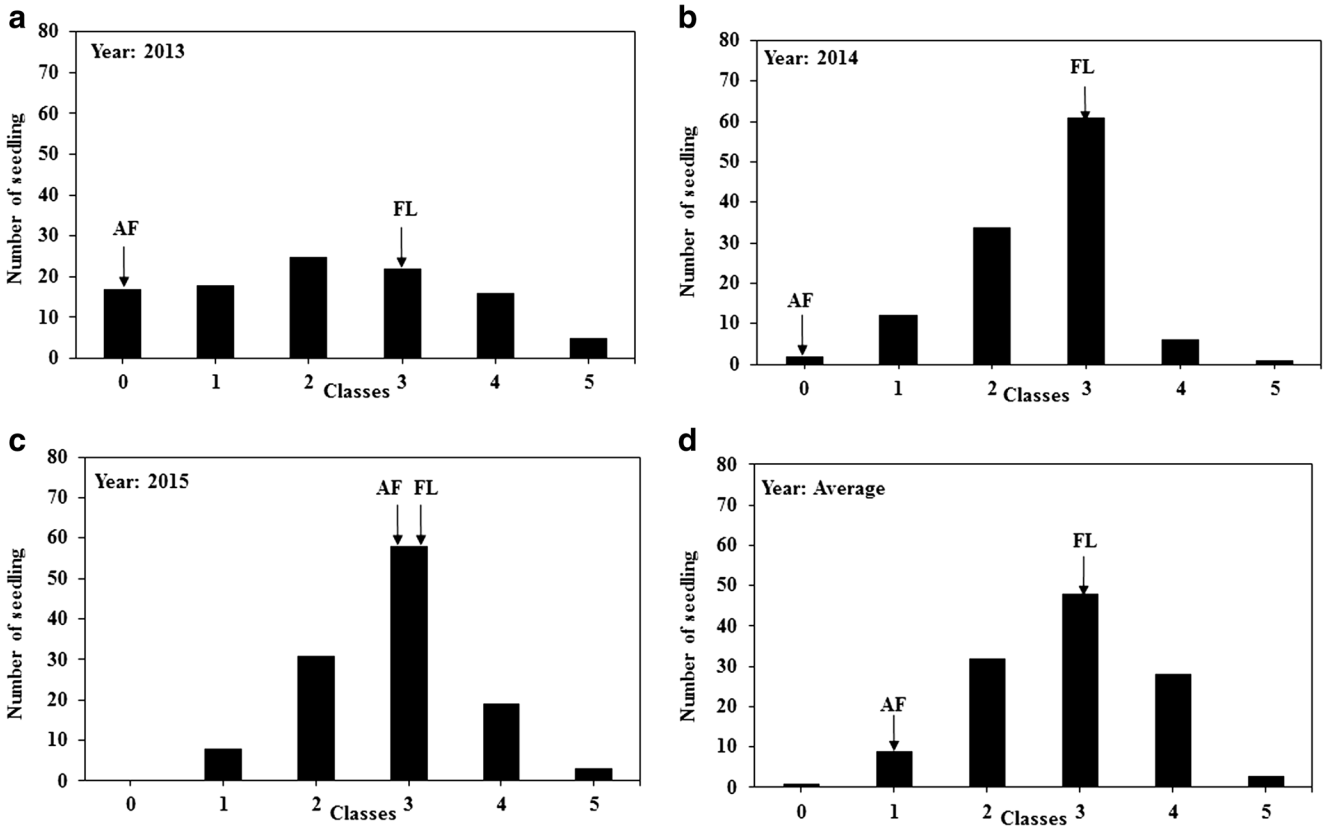


Fig. 2 Distribution of the percentage of the blush skin in the pear progeny of 'Flamingo' × 'Abate Fetel'. (**a**) 2013, (**b**) 2014, (**c**) 2015 and (**d**) average across all years. Arrows indicate parental scores: FL, 'Flamingo'; AF, 'Abate Fetel'

Inheritance of flowering time

The flowering time based on Julian dates at 80% full bloom over a period of 2 years (2014, 2015) as well as average data of the two seasons, in the progeny of 'Flamingo' × 'Abate Fetel', is shown in Fig. 4. In 2014, some seedlings reached 80% full bloom from 10 to 16 days before the parents, and others reached 80% full bloom 20 to 26 days later than the parents. In 2015, some seedlings reached 80% full bloom 9 days earlier than the parents did and some seedlings reached 80% full bloom 20 days later than the parents did. Seedlings, which flowered earlier or later than both parents in all years, showed transgressive segregation which could be indicative of polygenic control for flowering date or could be attributed to the different locations of the parents and seedlings. Average accumu

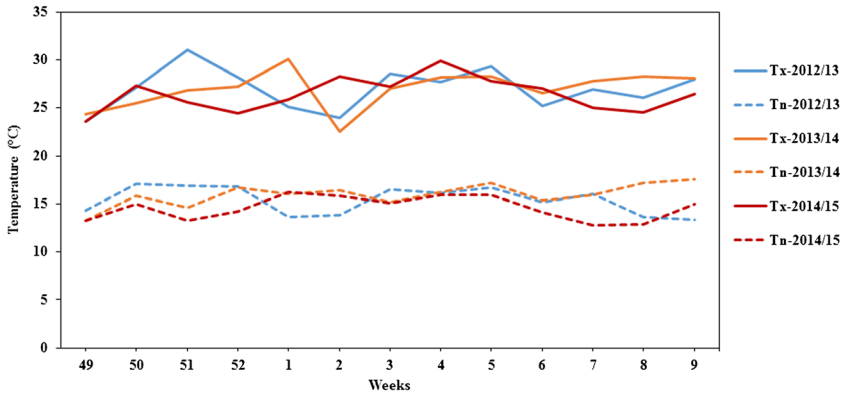


Fig. 3 Summary of the weather conditions from December 2012 to February 2013 (2012/13), December 2013 to February 2014 (2013/14) and December 2014 to February 2015 (2014/15) from the Drosternes Research Farm (34.04314° S, 19.04365° E) where the progeny of 'Flamingo' × 'Abate Fetel' was planted. During 2013, phenotyping was done from weeks 7 to 9, during 2014 from weeks 3 to 6 and during 2015 from weeks 1 to 3. Tn represents daily minimum temperature, Tx represents daily maximum temperature. The data were collected from the Riviera weather station (34.056233° S, 19.132233° E)

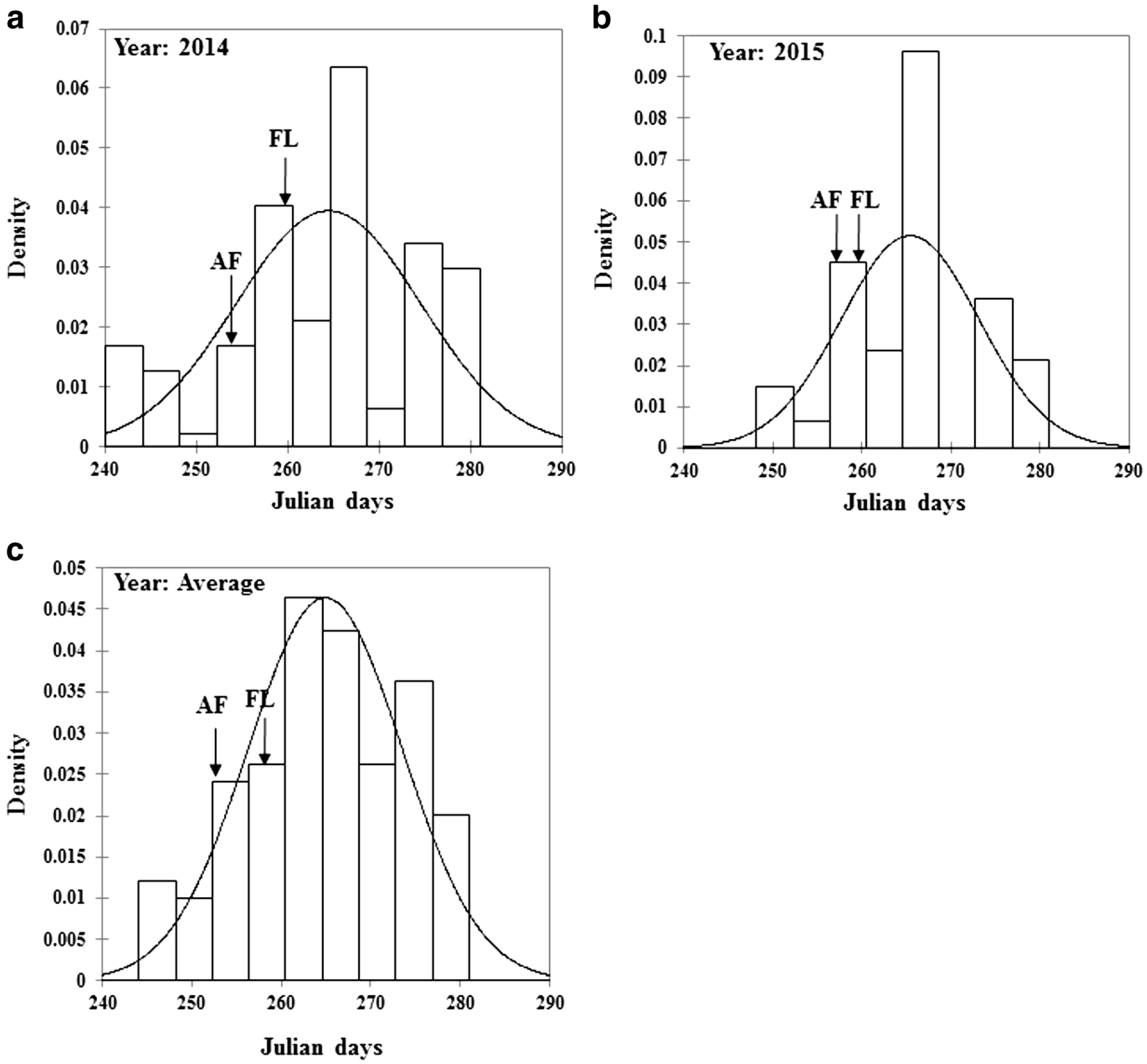


Fig. 4 Distribution for flowering time (80% full bloom) scored in Julian days in the pear progeny of 'Flamingo' × 'Abate Fetel'. (**a**) 2014, (**b**) 2015 and (**c**) average of 2014 and 2015. Arrows indicate parental values: FL, 'Flamingo': AF, 'Abate Fetel'

lated cold units (Fig. 5a) were recorded from the beginning of May to the end of August for each year (2014 and 2015), and average accumulated heat units (Fig. 5b) were recorded from the beginning of September to the end of October in both years. The results showed that the seedlings received fewer cold, but more heat units in 2014 than in 2015 (Fig. 5). Suprisingly, seedlings reached 80% full bloom earlier in the 2014 season than in 2015.

According to the Kolmogorov-Smirnov test, the 2014 data (Fig. 4a) and average data set (Fig. 4c) were normally distributed with $P = 0.184$ and $P = 0.476$, respectively, but the 2015 data set (Fig. 4b) was not normally distributed. However, the absolute value for the kurtosis test of this data set showed an absolute positive value of 0.718, larger than the absolute value for skewness of 0.107, indicating normal distribution. Thus, the data sets were suitable for QTL analysis. The repeatability was calculated as 0.78 based on all genotypes scored over 2 years, suggesting that scoring was consistent over the years.

SSR marker segregation

Of the 189 primer pairs tested, 28 amplified non-segregating alleles, four produced heterozygous alleles but were not used for mapping, 16 failed to amplify, one produced complex amplification patterns difficult to score and three segregated in a 3:1 ratio not usable for mapping (Table S4, Supplementary Material). The remaining 137 primer pairs were used for mapping. Twenty primer pairs amplified two or more loci resulting in 23 additional loci, which brings the total to 160 loci that were used to construct the genetic map (Table S5, Supplementary Material).

Construction of linkage maps

A total of 125 loci out of 160 were successfully mapped on the 'Flamingo' linkage map, which covered all 17 LGs with a total length of 973.8 cM and average distance of 9.5 cM between

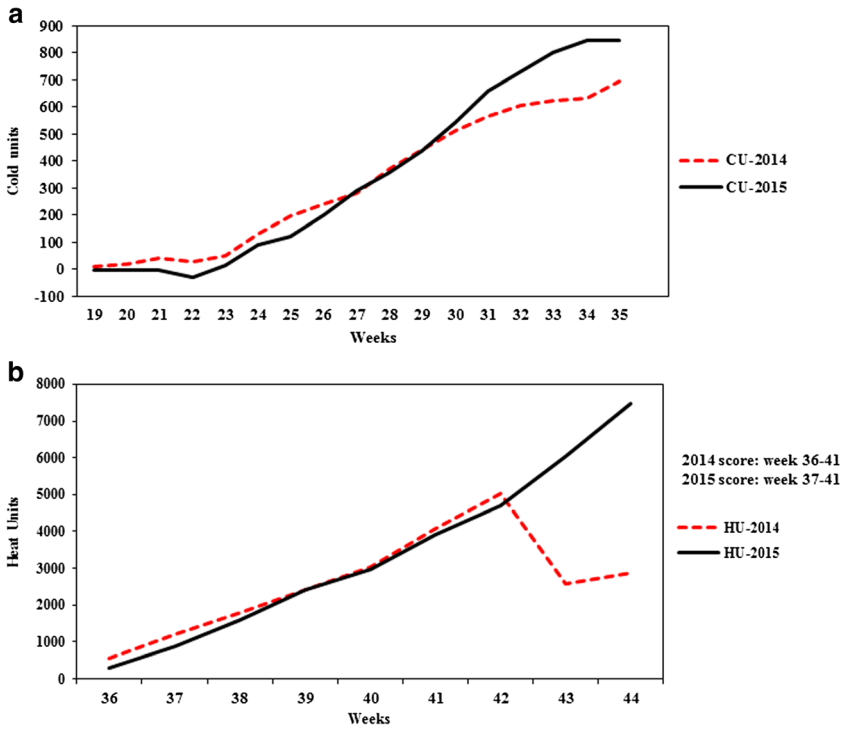


Fig. 5 Summary of the weather conditions based on average accumulated cold (**a**) and heat units (**b**) from the Drosternes Research Farm (34.04314°S, 19.04365° E) where the progeny of 'Flamingo' × 'Abate Fetel' was planted. The data were collected from the Riviera weather station (34.056233° S, 19.132233° E). HU, heat units; CU, chill units

markers (Fig. 6). Construction of this map allowed positioning of 10 previously unmapped loci, namely CN494669-z on LG10, CN857658-x on LG9, CN896931-z on LG4, CN907419-z on LG15, CN997047 on LG5, EMPc10-z on LG15, Hi01c11-z on LG8, Hi01c11-y on LG8, NH021a-z on LG4 and NH041a-z on LG6. The minimum number of markers mapped per LG was five (LG1, LG12, LG13), and the maximum number of markers mapped per LG was 14 (LG5). There were 10 gaps exceeding 20 cM in 10 LGs.

The 'Abate Fetel' map was constructed with 118 markers, which covered all 17 LGs and had a total length of 984.40 cM and average distance of 11.77 cM between markers (Fig. 6). This map allowed the positioning of 10 previously unmapped loci, namely CN857658-x on LG9, CN857658-w on LG2, EMPc10-w on LG5, EMPc10-y on LG15, EMPc10-z on LG15, Naupy32e-z on LG6, Naupy58b-z on LG7, NH013a-z on LG10, NH201a-z on LG5 and TsuGNH187-z on LG11. The minimum number of markers per LG was three (LG1), and the maximum number of markers per LG was 13 (LG9). There were 11 gaps exceeding 20 cM in 10 LGs.

Duplicated loci in 'Flamingo' × 'Abate Fetel'

A total of 20 primer pairs amplified more than one locus in the 'Flamingo' × 'Abate Fetel' progeny (Table S5, Supplementary Material). Eleven of these 20 primer pairs amplified two or three loci, namely CN494669, CN896931, CN907419, EMPc10, Naupy32e, Naupy46b, Naupy58b, NH013a, NH021a-1, NH041a and TsuGNH187. Eight primer pairs, CH02a08, CH04g09, CN857658, EMPc107, NB110a, NB119b, NB133a and NZmsEB107305, were previously reported to amplify two loci (Fernández-Fernández et al. 2008, 2012; Yamamoto et al. 2013; Terakami et al. 2014). One of the primer pairs, Hi01c11, amplified four loci, which mapped to three different LGs (Yamamoto et al. 2013; Terakami et al. 2014).

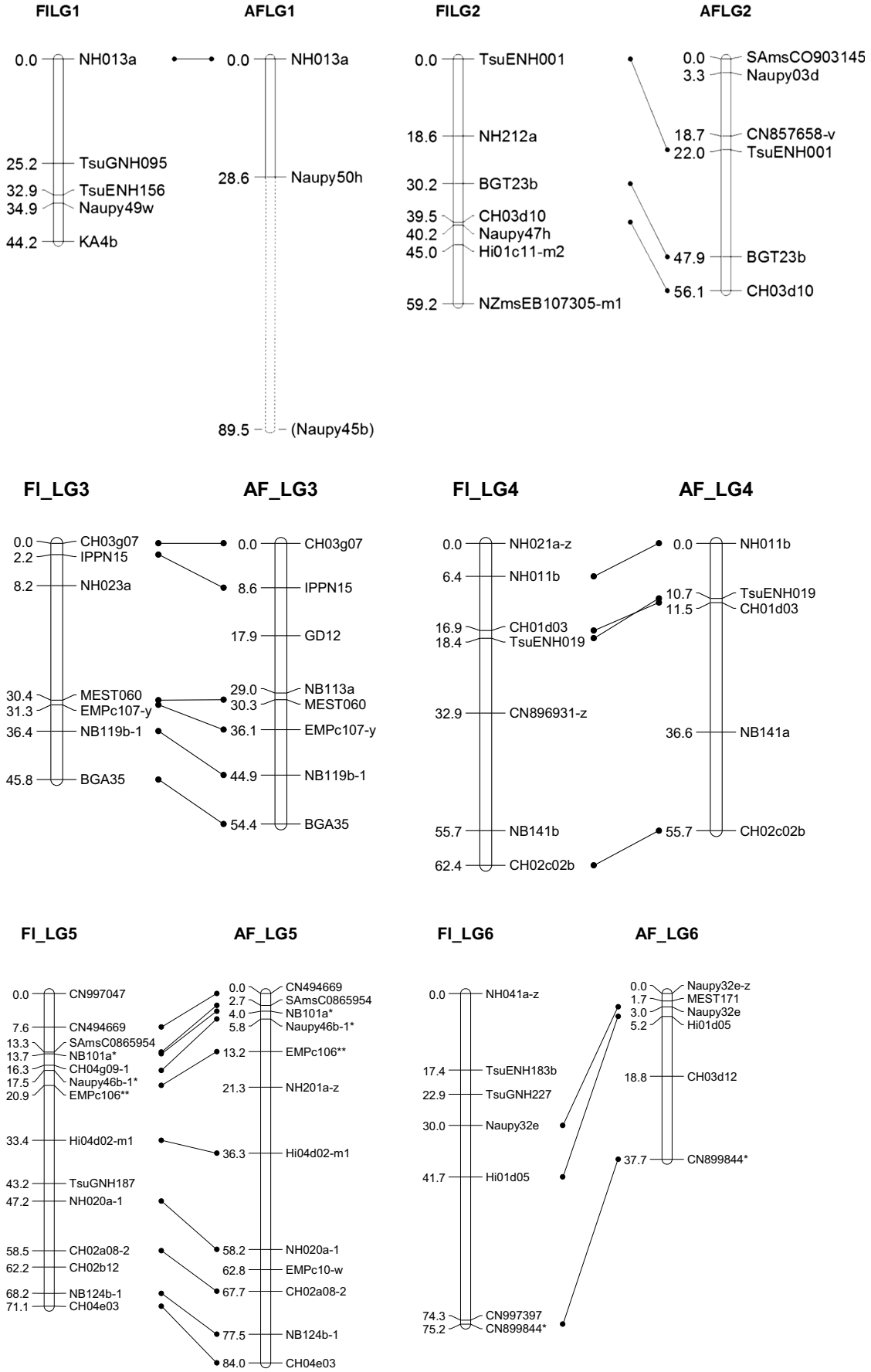
Two of the 11 loci that amplified two or three loci, namely Naupy32e and Naupy58b, mapped to the same LG, and for the remaining nine primer pairs, the loci mapped to different LGs. Primer pair EMPc10 amplified three loci that mapped to two different LGs.

Four pear primer pairs, namely NB124b, RLG1, TsuENH004 and TsuENH011, and three apple primer pairs, namely GD112, Hi0ld05 and Hi03a03, amplified a single locus even though they were previously reported to amplify duplicated loci.

QTL detection for blush skin

The genome-wide LOD threshold was estimated at 3.1–3.2. Restricted Multiple QTL Method (rMQM) mapping for blush skin detected two significant QTLs, one at the telomeric region of LG5 in 'Flamingo' and the other at the telomeric region of LG9 in 'Abate Fetel' (Table 3).

Fig. 6 Genetic linkage maps constructed from the 'Flamingo' × 'Abate Fetel' progeny. Fl represents the 'Flamingo' map with 125 SSR loci and AF represents the 'Abate Fetel' map with 119 SSR loci. Distorted segregation ratios: $*P < 0.05$, $**P < 0.01$ and $****P < 0.0001$. Homologous loci are connected with black lines. The isolated locus Naupy45b on LG1 in the 'Abate Fetel' map was inserted based on its position in published maps


FILG1
0.0 NH013a
25.2 TsuGNH095
32.9 TsuENH156
34.9 Naupy49w
44.2 KA4b
AFLG1
0.0 NH013a
28.6 Naupy50h
89.5 (Naupy45b)
FILG2
0.0 TsuENH001
18.6 NH212a
30.2 BGT23b
39.5 CH03d10
40.2 Naupy47h
45.0 Hi01c11-m2
59.2 NZmsEB107305-m1
AFLG2
0.0 SAmsCO903145
3.3 Naupy03d
18.7 CN857658-v
22.0 TsuENH001
47.9 BGT23b
56.1 CH03d10
FI_LG3
0.0 CH03g07
2.2 IPPN15
8.2 NH023a
30.4 MEST060
31.3 EMPc107-y
36.4 NB119b-1
45.8 BGA35
AF_LG3
0.0 CH03g07
8.6 IPPN15
17.9 GD12
29.0 NB113a
30.3 MEST060
36.1 EMPc107-y
44.9 NB119b-1
54.4 BGA35
FI_LG4
0.0 NH021a-z
6.4 NH011b
16.9 CH01d03
18.4 TsuENH019
32.9 CN896931-z
55.7 NB141b
62.4 CH02c02b
AF_LG4
0.0 NH011b
10.7 TsuENH019
11.5 CH01d03
36.6 NB141a
55.7 CH02c02b
FI_LG5
0.0 CN997047
7.6 CN494669
13.3 SAmsC0865954
13.7 NB101a*
16.3 CH04g09-1
17.5 Naupy46b-1*
20.9 EMPc106**
33.4 Hi04d02-m1
43.2 TsuGNH187
47.2 NH020a-1
58.5 CH02a08-2
62.2 CH02b12
68.2 NB124b-1
71.1 CH04e03
AF_LG5
0.0 CN494669
2.7 SAmsC0865954
4.0 NB101a*
5.8 Naupy46b-1*
13.2 EMPc106**
21.3 NH201a-z
36.3 Hi04d02-m1
58.2 NH020a-1
62.8 EMPc10-w
67.7 CH02a08-2
77.5 NB124b-1
84.0 CH04e03
FI_LG6
0.0 NH041a-z
17.4 TsuENH183b
22.9 TsuGNH227
30.0 Naupy32e
41.7 Hi01d05
74.3 CN997397
75.2 CN899844*
AF_LG6
0.0 Naupy32e-z
1.7 MEST171
3.0 Naupy32e
5.2 Hi01d05
18.8 CH03d12
37.7 CN899844*

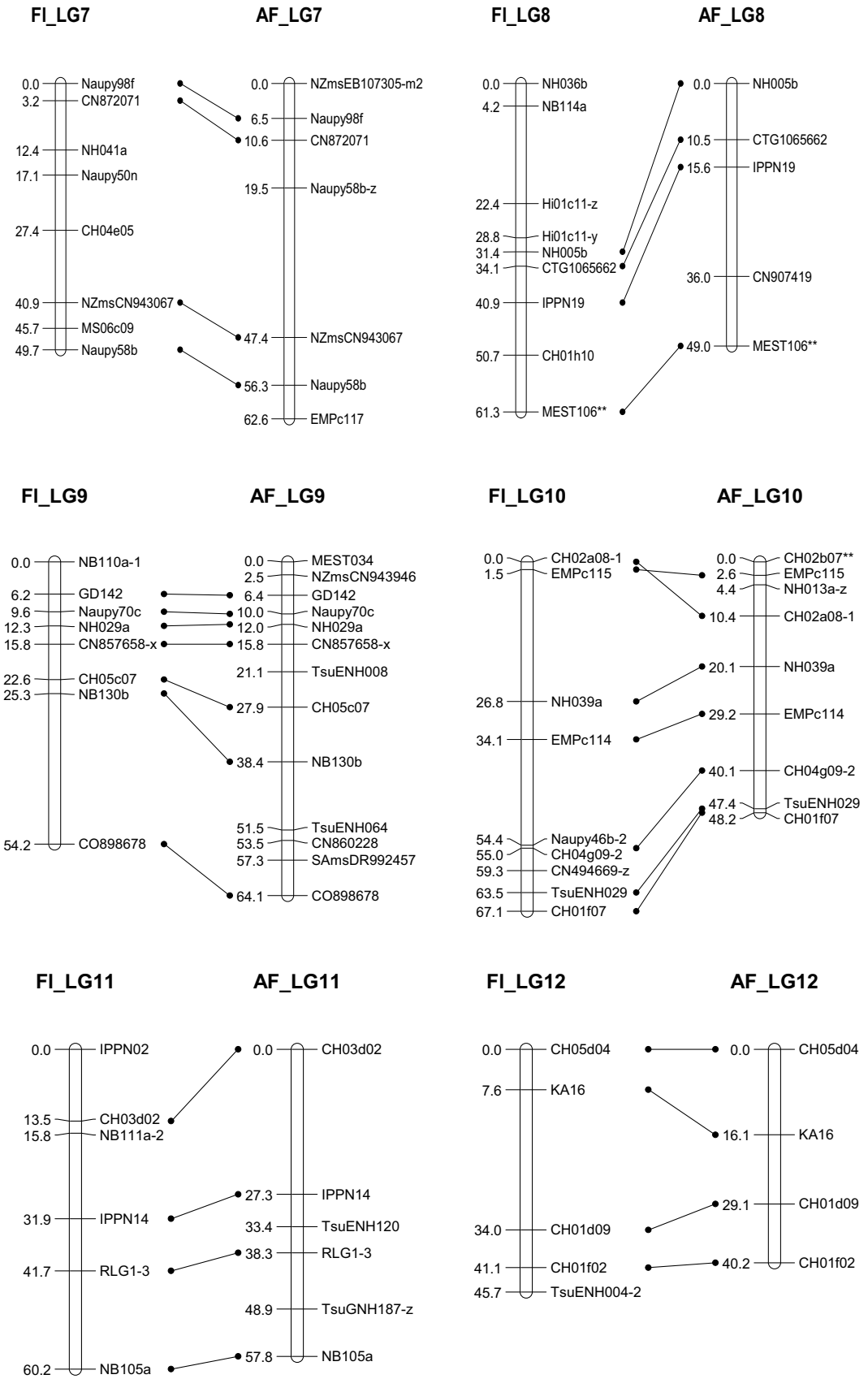


Fig. 6 continued.

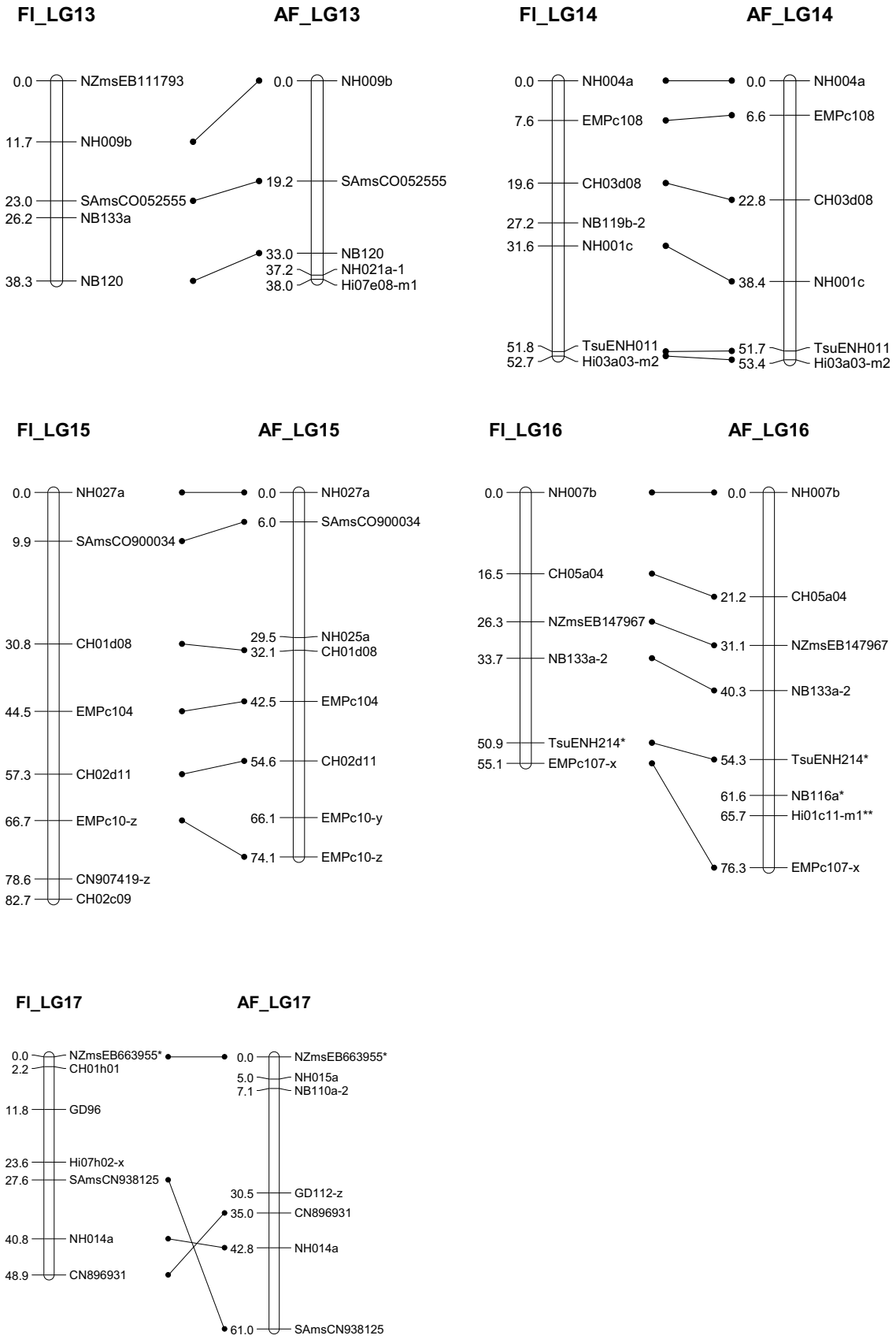


Fig. 6 continued.

Table 3 QTLs identified for blush skin and flowering time in the progeny of 'Flamingo' × 'Abate Fetel' pear using restricted MQM (rMQM) mapping. QTLs with LOD scores above the genome-wide LOD score and explaining more than 10% phenotypic variance are indicated in bold. QTLs with LOD scores below the genome-wide LOD score and explaining less than 10% phenotypic variance are indicated in italics

Trait	Data set	LOD threshold—genome-wide	LG	Parental map	QTL interval (cM)	Marker closest to peak	Position (cM)	LOD marker	%Var
Blush	2013	3.2	5	Flamingo	7.6–16.3	SAmsCO865954	13.3	**4.68**	**18.9**
	2014	2.9	9	Abate Fetel	2.5–10.0	GD142	6.4	**3.98**	**14.6**
	2014	3.1	5	Flamingo	7.6–16.3	SAmsCO865954	13.3	**4.09**	**15.0**
	2015	3.0	9	Abate Fetel	10.0–15.8	NH029a	12.0	*2.46*	*9.1*
	2015	2.9	5	Flamingo	7.6–16.3	NB101a	13.7	**5.81**	**23.2**
	Average	3.1	9	Abate Fetel	10.0–15.0	NH029a	12.0	*2.69*	*9.7*
	Average	3.2	5	Flamingo	7.6–16.3	SAmsCO865954	13.3	**7.99**	**26.2**
Flowering time	2014	3.1	9	Flamingo	0.0–9.6	GD142	6.2	**7.88**	**27.1**
	2015	3.1	9	Abate Fetel	0.0–2.5	MEST034	0.0	**6.83**	**15.9**
	2015	3.1	9	Flamingo	0.0–9.6	GD142	6.2	**13.29**	**35.7**
	Average	3.2	9	Abate Fetel	0.0–2.5	MEST034	0.0	**4.48**	**11.3**
	Average	3.2	9	Flamingo	0.0–9.6	GD142	6.2	**12.07**	**35.9**

In 'Flamingo', a QTL was detected on LG5 across all 3 years (2013, 2014 and 2015) and in the average data set (Fig. 7). Marker SAmsCO865954 on LG5 was found to be the closest marker to the QTL peak and had a LOD score above the genome-wide LOD threshold across all years and based on average data set, ranging from 4.09 to 7.99. This marker explained between 15.0 and 26.5% of the phenotypic variance. Marker NB101a that was ~ 0.4 cM from SAmsCO865954 was also close to the QTL peak with the LOD score above the genome-wide LOD threshold across all years and based on average data set, ranging from 4.09 to 7.65 and explaining 15.0 to 25.3% of the phenotypic variance. This QTL was not detected when the data set was grouped in no blush (0) versus blushed (1, 2, 3, 4, 5), but was confirmed in both groupings of 0 and 1 (no blush) versus 2, 3, 4 and 5 (blush) and 0, 1 and 2 (no to light blush) versus 3, 4 and 5 (blush). The QTL on LG5 in 'Flamingo' was detected in the grouping of 0 and 1 versus 2, 3, 4 and 5 based on the 2013, 2014 and average data sets (Table 4; Fig. S1). Markers SAmsCO865954 and Naupy46b-1 were closest to the QTL peak with the LOD scores ranging from 2.11 to 3.31 above the genome-wide threshold score. These markers explained 7.7 to 13.7% of the phenotypic variance. The grouping of 0, 1 and 2 versus 3, 4 and 5 detected the QTL on LG5 of 'Flamingo' based on the 2013, 2014, 2015 and average data sets (Table 4). Markers, SAmsCO865954 and NB101a, were closest to the QTL peak with the LOD scores ranging from 4.49 to 6.32 above

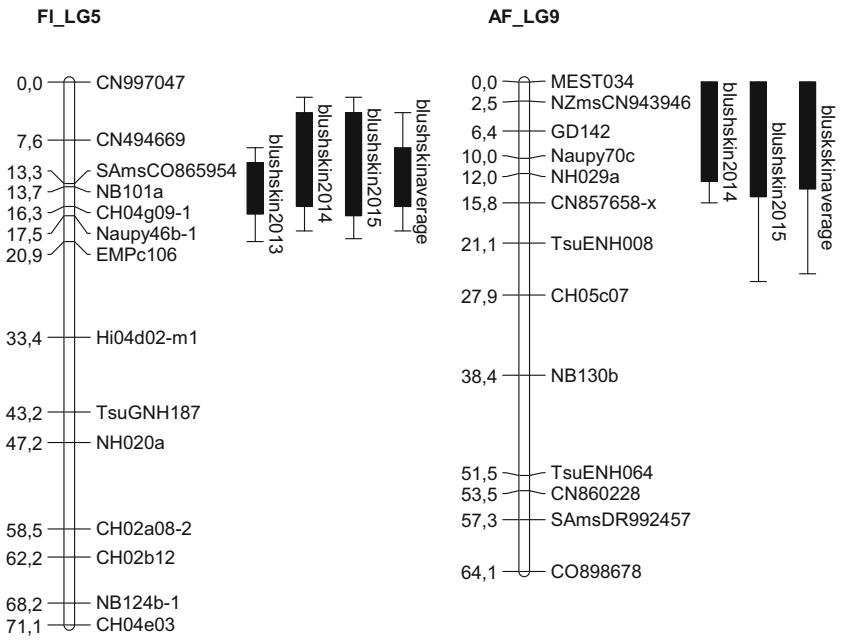


Fig. 7 Position of the QTLs for blush skin detected on LG5 in 'Flamingo' and LG9 in 'Abate Fetel' in the pear progeny of 'Flamingo' × 'Abate Fetel'. Black boxes represent QTLs where length of the boxes corresponds to 1-LOD confidence intervals and extended lines represent 2-LOD confidence intervals

Table 4 QTLs identified for blush skin in the progeny of 'Flamingo' × 'Abate Fetel' pear based on grouping of different classes scored used to test the 5:3 segregation model using restricted MQM mapping (rMQM). QTLs with LOD scores above the genome-wide LOD score and explaining more than 10% phenotypic variance are indicated in italics

Trait	Classes	Data set	LOD threshold—genome-wide	LG	Parental map	QTL interval (cM)	Marker closest to peak	Position (cM)	LOD marker	%Var
Blush (0, 1 vs 2, 3, 4, 5)		2013	3.1	5	Flamingo	7.6–16.3	SAmsCO865954	13.3	*3.28*	*13.7*
		2014	2.9	5	Flamingo		Naupy46b-1		*3.31*	*12.3*
		Average	2.7	5	Flamingo		Naupy46b-1		*2.11*	*7.7*
Blush (0, 1, 2 vs 3, 4, 5)		2013	3.0	5	Flamingo	7.6–16.3	SAmsCO865954	13.3	*4.53*	*18.3*
		2014	3.0	9	Abate Fetel		GD142		*3.75*	*12.8*
		2014	3.0	5	Flamingo		NB101a		*4.49*	*16.3*
		2015	3.0	5	Flamingo		NB101a		*6.32*	*21.7*
		Average	3.0	9	Abate Fetel		NH029a		*2.7*	*9.8*
		Average	3.0	5	Flamingo		SAmsCO865954		*5.8*	*19.8*

the genome-wide threshold scores and explained 18.3 to 21.7% of the phenotypic variance (Table 4; Fig. S1).

In 'Abate Fetel', one QTL was detected with rMQM at the telomeric region of LG9 (Fig. 7) based on the data sets scored in 2014, 2015 and the average data sets. The analysis of the 2014 data set showed that marker GD142 on LG9 in 'Abate Fetel' was close to the peak with a LOD score of 3.98 above the genome-wide threshold LOD score and explained 14.6% of the phenotypic variance. In the analysis of the 2015 data set and the average data set, marker NH029a, located in the upper telomeric region of LG9 in 'Abate Fetel', was the closest to the QTL peak. This marker had a LOD score that ranged from 2.46 to 2.69 below the genome-wide threshold LOD score and explained between 9.1 and 9.7% of the phenotypic variance. This QTL was not detected when the data was grouped into 0 (no blush) versus 1, 2, 3, 4 and 5 (blushed), or 0 and 1 (no blush) versus 2, 3, 4 and 5 (blushed) but was detected in the grouping of 0, 1 and 2 (light blush) versus 3, 4 and 5 (blushed) based on the 2014 and average data sets. Markers, GD142 and NH029a, were found to be closest to the QTL peak with the LOD score of 3.75 (above the genome-wide threshold LOD score) and 2.7 (below the genome-wide threshold LOD score) and explained 9.8 to 12.8% of the phenotypic variance (Table 4; Fig. S1).

Alleles 139 and 462 inherited, respectively, from markers NB101a and SAmsCO865954, on LG5 scored in 'Flamingo' were associated with the blush skin QTL (Table 5). Statistical analysis of the two markers associated with the blush trait showed moderate occurrence of type I (α) and type II (β) errors across all 3 years of 20% or more (Table 6). These results also revealed that the markers showed sensitivity, power ($1-\beta$) and accuracy of more than 70%, and specificity of more than 65% in all 3 years. The predictive value negative was higher in 2013 at 80% compared to 2014 and 2015 and the predictive value positive was less in 2013 compared to 2014 and 2015 (Table 6). Analysis of these markers using rMQM revealed significant QLTs whether the markers were analysed individually or in combination.

In 'Abate Fetel', allele 89 (from marker NH029a) and allele 169 (from marker GD142) on LG9 were associated with the blush skin in 2 years (2014 to 2015) and the average data set (Table 5). The type I and type II errors of these markers varied between years. The type I error for these markers varied between 18.75% in 2014 and 23% in 2015 and the type II error varied between 20% in 2014 and 57.1% in 2015 (Table 6). These markers had high specificity and predictive value positive in both years, but showed variations in sensitivity, power ($1-\beta$) and accuracy between years. Significant QTLs were detected for both markers using rMQM whether markers were analysed individually or in combination.

Candidate gene identification for blush skin in pear

On apple LG5, 572 genes were found located within the chosen marker intervals of 13.3 cM between CH03a09 and NZmsEB132749 in the apple genome. Three candidate genes, which show homology to *MYB8*6, were located between 1464 and 1977 kbp from marker NB101a, which located between coordinates Chr05:39,560,707-39,626,652 on LG5 in apple (Table 7). These three genes have been shown to be orthologous to three pear genes. Four candidate genes of *UDP-glucosyl transferase* were also found on the apple LG5 located between 1321 and 1331 kbp from marker NB101a, closest to the QTL region. These four genes were orthologous to two pear genes.

On apple LG9, 2185 genes were found located within the chosen marker intervals of 23.9 cM between NZmsCN943946 and NZmsEB116209. Three candidate genes, showing homology to *MYB39*, occur between 213 and 630 kbp from marker GD142, mapped at 8.1 cM on the apple map (Fernández-Fernández et al. 2012). These three candidate genes were orthologous to two pear genes. Additionally, two candidate genes of *MYB21* occur between 357 and 358 kbp from marker GD142 on the apple LG5 and were found to be orthologous to one pear gene (Table 7).

Table 5 Inheritance of allele 139 (of marker NB101a) and allele 462 (of marker SAmsCO865954) on LG5 in 'Flamingo' and allele 89 (of marker NH029a) and allele 169 (of marker GD142) on LG9 in 'Abate Fetel' and their association with the blush skin QTL based on the 2013, 2014, 2015 and average (of all 3 years) data sets. Calculated percentage (%) indicates the distribution of allele inheritance within a specific blush phenotypic class

	Seedlings with allele 139, 462	Seedlings without allele 139, 462	Total	Seedlings with allele 89, 169	Seedlings without allele 89, 169	Total
Year 2013						
Class 0	5 (29%)	12 (71%)	17			
Class 1	4 (22%)	14 (78%)	18			
Class 2	11 (44%)	14 (56%)	25			
Class 3	15 (68%)	7 (32%)	22			
Class 4	14 (87.5%)	2 (12.5%)	16			
Class 5	4 (80%)	1 (20%)	5			
Year 2014						
Class 0	1 (50%)	1 (50%)	2	0 (0%)	2 (100%)	2
Class 1	1 (8%)	11 (92%)	12	2 (17%)	10 (83%)	12
Class 2	13 (38%)	21 (62%)	34	9 (26%)	25 (74%)	34
Class 3	44 (72%)	17 (28%)	61	34 (64%)	27 (44%)	61
Class 4	4 (67%)	2 (33%)	6	4 (67%)	3 (33%)	7
Class 5	1 (100%)	0 (0%)	1	1 (100%)	0 (0%)	1
Year 2015						
Class 0	0 (0%)	0 (0%)	0	0 (0%)	0 (0%)	0
Class 1	2 (25%)	6 (75%)	8	2 (25%)	6 (75%)	8
Class 2	6 (19%)	25 (81%)	31	9 (29%)	22 (71%)	33
Class 3	36 (62%)	22 (38%)	58	27 (47%)	31 (53%)	58
Class 4	17 (89.5%)	2 (10.5%)	19	16 (84%)	3 (16%)	19
Class 5	3 (100%)	0 (0%)	3	1 (33%)	2 (67%)	3
Average						
Class 0 (0–0.49)	1 (100%)	0 (0%)	1	0 (0%)	1 (100%)	1
Class 1 (> 0.5–1)	1 (11.1%)	8 (88.9%)	9	2 (22.2%)	7 (77.8%)	9
Class 2 (1.01–2.0)	8 (25%)	24 (75%)	32	7 (21.9%)	25 (78.1%)	32
Class 3 (2.01–3)	30 (62.5%)	18 (37.5%)	48	20 (41.7%)	28 (58.3%)	48
Class 4 (3.01–4)	22 (78.6%)	6 (21.4%)	28	18 (64.3%)	10 (35.7%)	28
Class 5 (4.01–5)	3 (100%)	0 (%)	3	2 (66.7%)	1 (33.3%)	3

QTL detection for flowering time

QTL analysis for flowering time based on the 2014, 2015 and the average data sets located a QTL on LG9 in both parents (Fig. 8). In 'Flamingo', analysis of the 2014 and 2015 data sets and the average data set showed that marker GD142 was closest to the peak with a LOD score ranging from 7.88 to 13.29 (which exceeded the genome-wide threshold LOD score ranging from 3.1 to 3.2). This QTL explained between 27.7 and 35.9% of the phenotypic variance (Table 3). In 'Abate Fetel', QTL detection revealed the location of a putative QTL at the telomeric region of LG9 based on the 2015 data set and the average data set. Marker MEST034 was closest to the peak with a LOD score ranging from 4.48 to 6.83 (above the genome-wide threshold LOD score) and explaining between 11.3 and 15.9% of the phenotypic variance. This QTL was not detected in the 2014 data set. Table 8 shows alleles 161 and 167 of marker GD142 in 'Flamingo' associated with flowering time. Allele 161 seems to be associated with early to medium flowering time over 2 years (2014 to 2015) and in the average data set whereas allele 167 seems to be associated with late flowering.

Statistical analysis of marker GD142 with allele 161 associated with early flowering showed that type I (α) error varied from 13.6 to 30.1% and type II (β) error from 12.9 to 19% across the years (Table 9). This marker had a sensitivity and power ($1-\beta$) of more than 80%, specificity of about 70% and accuracy of more than 70% in both years. This statistical analysis also revealed variation between predictive value negative and predictive value positive between the years. A QTL for flowering time detected in 'Abate Fetel' showed that neither of the alleles 159 and 169 of GD142 appeared to be associated with early flowering in 'Abate Fetel'.

Table 6 Statistical summary of markers NB101a (allele 139) and SAmsCO865954 (allele 462) on LG5 in 'Flamingo' and markers GD142 (allele 169) and NH029a (allele 89) on LG9 in 'Abate Fetel' associated with blush skin as marker-assisted selection tool using the Hühn and Piepho (2003) system. Probability quantification is based on percentages (%)

Year 2013	NB101a/SAmsCO865954 in 'Flamingo'
Type I error (α)	33
Type II error (β)	23
Power ($1-\beta$)	77
Sensitivity	77
Specificity	66.7
Predictive value positive (PVP)	62.3
Predictive value negative (PVN)	80
Accuracy	70.9
Year 2014	NB101a/SAmsCO865954 in 'Flamingo'
Type I error (α)	31.3
Type II error (β)	27.9
Power ($1-\beta$)	72.1
Sensitivity	72
Specificity	68.8
Predictive value positive (PVP)	76.6
Predictive value negative (PVN)	63.5
Accuracy	70.7
Year 2015	NB101a/SAmsCO865954 in 'Flamingo'
Type I error (α)	20.5
Type II error (β)	30
Power ($1-\beta$)	70
Sensitivity	70
Specificity	79.5
Predictive value positive (PVP)	87.5
Predictive value negative (PVN)	56.4
Accuracy	73.1
Year 2014	NH029a/GD142 in 'Abate Fetel'
Type I error (α)	18.75
Type II error (β)	20
Power ($1-\beta$)	80
Sensitivity	52.9
Specificity	81.25
Predictive value positive (PVP)	80
Predictive value negative (PVN)	54.9
Accuracy	64.7
Year 2015	NH029a/GD142 in 'Abate Fetel'
Type I error (α)	23
Type II error (β)	57.1
Power ($1-\beta$)	42.9
Sensitivity	50
Specificity	76.9
Predictive value positive (PVP)	81.6
Predictive value negative (PVN)	42.9
Accuracy	58.8

Validation of blush skin associated markers in other pear cultivars and selections

To validate the utility of the markers NB101a and SAmsCO865954 with alleles 139 and 462, respectively, associated with blush skin on LG5, 25 pear accessions were genotyped with these markers (Table 2). The desirable threshold score for blush pear at the ARC pear breeding programme ranges from medium blush (class 3) to excellent blush (class 5). Sixteen of the 25 accessions derive from 'Forelle' or are derivatives of 'Forelle' (one of the parents of 'Flamingo'), of which 11 accessions had both alleles: five accessions scored as medium (class 3) to good blush (class 4), three accessions scored as light blush (class 2) and three accessions scored as no blush to very light blush (classes 0 and 1). The remaining five 'Forelle'-derived accessions had none of these alleles, of which three accessions were scored as medium blush (class 3) to good blush (class 4), one accession scored as light blush (class 2) and one accession had very light blush (class 1). The other seven accessions not related to 'Forelle' scored as medium blush (class 3) to purple red (class 6) and lacked both alleles with the exception of cultivars 'Celina' and 'Hortensia' and selection P07-3 having allele 462. A further two accessions also not related to 'Forelle' also lacked both alleles. One scored as very light bush (class 1) and the other scored as light blush (class 2).

Discussion

Analysis of the phenotypic traits

Visual scoring of blush skin based on a 6-point scale over 3 years was found useful to evaluate the segregation of this trait. 'Flamingo' displayed constant scores for blush skin across all years whereas 'Abate Fetel' showed variation. The constant scores displayed by 'Flamingo' are associated with continuous anthocyanin synthesis despite high temperatures (Steyn et al. 2004a, b). The data set scored during 2015 showed more blush compared to the data sets scored during the 2013 and 2014 seasons and this may have been attributed to differences in temperature affecting the expression of the blush genes. The 2015 season was cooler compared to the 2013 and 2014 seasons, which could have promoted the accumulation of anthocyanin compounds and resulted in good blush development. A previous study in pear by Steyn et al. (2004a, b) reported that high temperatures promoted degradation of anthocyanin accumulation in 'Forelle' and 'Rosemarie' and fading of red skin colouration in these cultivars. Low repeatability of the trait also highlights the large influence of the environment on the expression of the trait. The lack of more replicates of the seedlings in multiple envirionments, which would have allowed for the more

Table 7 *MYB* genes and genes associated with anthocyanin biosynthesis in LG5 and LG9 in the apple genome around the QTL regions associated with blush skin in the pear progeny of 'Flamingo' × 'Abate Fetel'

Gene name	Apple gene ID	Pear gene ID	LG	Marker closest to QTL	Distance from marker (kbp)	Function
MYB86	MDP0000289671	PCP010020.1	LG5	NB101a	1464	Transcription factor
MYB86	MDP0000157506	PCP023090.1	LG5	NB101a	1974	Transcription factor
MYB86	MDP0000158937	PCP037831.1	LG5	NB101a	1977	Transcription factor
UDP-glucosyl transferase 71C4	MDP0000242552	PCP010010.1 PCP010011.1	LG5	NB101a	1321	Codes anthocyanin enzyme
UDP-glucosyl transferase 71C3	MDP0000122122		LG5	NB101a	1322	Codes anthocyanin enzyme
UDP-glucoronosyl/ glucosyl transferase	MDP0000437996		LG5	NB101a	1327	Codes anthocyanin enzyme
UDP-glucosyl transferase 71B6	MDP0000437997		LG5	NB101a	1331	Codes anthocyanin enzyme
MYB39	MDP0000278681	PCP000135.1	LG9	GD142	630	Transcription factor
MYB39	MDP0000121476	PCP025136.1	LG9	GD142	610	Transcription factor
MYB39	MDP0000228930		LG9	GD142	213	Transcription factor
MYB21	MDP0000250031	PCP026600.1	LG9	GD142	357	Transcription factor
MYB21	MDP0000305363		LG9	GD142	358	Transcription factor

accurate evaluation of the genetic effects, might have limited the efficient assessment of this trait.

Segregation of blush skin in the current study in this progeny of 121 seedlings was not consistent with the previously reported segregation ratio of 5:3 (Human 2013). The discrepancies observed between the two studies could be attributed to the different scoring systems and possible unknown parentage of the seedlings used by Human (2013). The current study scored the trait on several occasions during the ripening season while Human (2013) scored the trait only once. Human (2013) also did not verify the trueness to parentage of the progeny that was studied. The variation observed within and between seasons indicates that the trait is polygenic. This was confirmed by QTL analysis based on the grouping of the different classes which revealed two QTLs, one on LG5 in 'Flamingo' and another one on LG9 in 'Abate Fetel'. These results revealed that blush trait is quantitative and clearly not in accord with the proposed two-gene model proposed by Human (2013). Phenotyping of the trait by means of the 6-point scale could have limited the detection of QTLs, due to

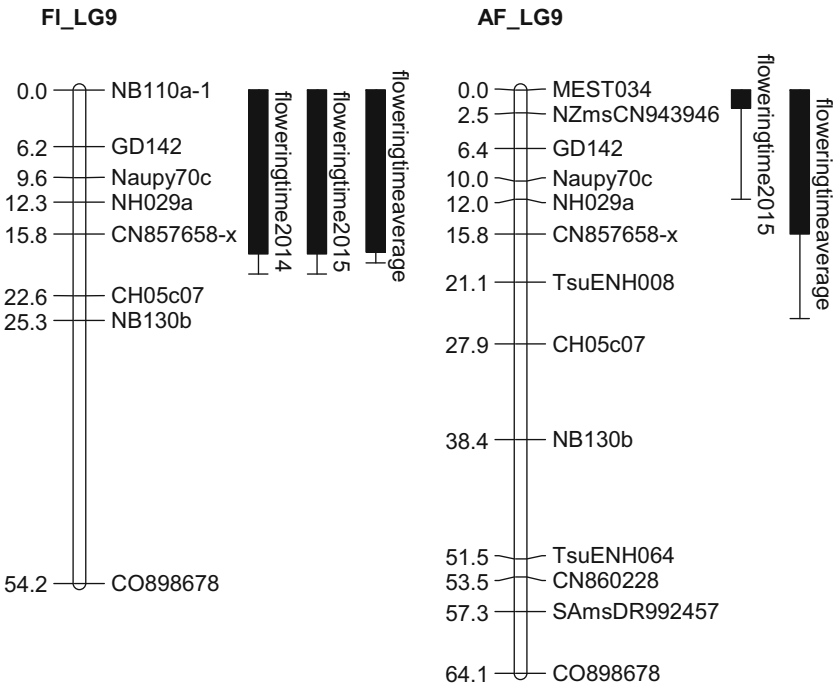


Fig. 8 Position of the QTLs for flowering time detected on LG9 in 'Flamingo' and 'Abate Fetel' in the pear progeny of 'Flamingo' × 'Abate Fetel'. Black boxes represent QTLs where length of the boxes corresponds to 1-LOD confidence intervals and extended lines represent 2-LOD confidence intervals

Table 8 Inheritance of alleles 161 and 167 from marker GD142 on LG9 in 'Flamingo' coupled with the QTL associated with early to medium flowering time based on Julian date data scored during 2014, 2015 and average (of both years) data set. Calculated percentage (%) indicates the distribution of allele inheritance within a specific flowering phenotypic class

Julian date	Seedlings with allele 161	Seedlings with allele 167	Total
Year 2014			
241–250	10 (71%)	4 (29%)	14
251–260	24 (86%)	4 (14%)	28
261–270	19 (47.5%)	21 (52.5%)	40
271–280	3 (9%)	30 (91%)	33
Year 2015			
241–250	4 (100%)	0 (0%)	4
251–260	23 (85%)	4 (15%)	27
261–270	23 (41%)	33 (59%)	56
271–280	2 (7%)	25 (93%)	27
Average			
241–250	7 (87.5%)	1 (12.5%)	8
251–260	23 (82%)	5 (18%)	28
261–270	24 (47%)	27 (53%)	51
271–280	3 (9%)	31 (91%)	34

the quantitative nature of this trait, and higher resolution of the phenotyping, such as measuring anthocyanin content, will aid in better QTL detection.

Table 9 Statistical summary of marker GD142 with allele 161 on LG9 in 'Flamingo' associated with early flowering as marker-assisted selection tool using the Hühn and Piepho (2003) system. Probability quantification are based on percentages (%)

Year	GD142
2014	
Type I error (α)	13.6
Type II error (β)	19
Power ($1-\beta$)	81
Sensitivity	81
Specificity	69.9
Predictive value positive (PVP)	60.7
Predictive value negative (PVN)	86.4
Accuracy	73.9
2015	
Type I error (α)	30.1
Type II error (β)	12.9
Power ($1-\beta$)	87.1
Sensitivity	87.1
Specificity	70
Predictive value positive (PVP)	52
Predictive value negative (PVN)	94
Accuracy	75

The scoring of flowering at 80% full bloom over 2 years was sufficient to allow for the study of the segregation of the trait. The observed transgressive segregation, in which some of the seedlings reached 80% full bloom earlier than either parent in both years, could be an indication of multiple genes controlling flowering time in this progeny or due to the different locations in which seedlings and parents were grown. Previous studies in Rosaceae species have also found that flowering time is controlled by several genes (Liebhard et al. 2003; Fan et al. 2010; Campoy et al. 2011; Kunihisa et al. 2014). The late blooming observed in the progeny of 'Flamingo' × 'Abate Fetel' during the 2015 season was attributed to the accumulation of high cold units and less heat units required to initiate flowering compared to the 2014 season, which accumulated sufficient cold units and heat units. These results appear to show that in the current study, heat unit accumulation potentially has a greater influence on flowering time than cold unit accumulation but this needs to be validated in future studies over more seasons.

Comparison of genetic maps

Comparing the 'Flamingo' and 'Abate Fetel' maps with previous European pear reference maps (Nishitani et al. 2009; Yamamoto et al. 2013) and an Asian pear reference map (Chen et al. 2015) found, as expected, that many of the common markers mapped in similar order and positions with just a few discrepancies such as some markers being reversed in positions. The maps of 'Bartlett' (Nishitani et al. 2009; Yamamoto et al. 2013) were constructed on the basis of 63 seedlings and the combined map of 'Bayuehong' × 'Dangshansuli' (Chen et al. 2015) was constructed from 51 seedlings, in comparison to the 121 seedlings used in the current study. The lower number of seedlings used for these original maps could have contributed to the minor inconsistencies.

QTL detection for blush skin

Two major QTLs, explaining > 10% of the phenotypic variance for blush skin, were detected in the 'Flamingo' × 'Abate Fetel' progeny. The major QTL detected on LG5 in 'Flamingo' explained more than 20% of the observed phenotypic variance and was consistent over all 3 years. A study in the Asian pear blush cultivar 'Bayuehong' by Yao et al. (2017) found a QTL associated with red skin colour on the same telomeric region of LG5 where the QTL was detected in the current study, suggesting that the same gene(s) might be responsible for red skin colouration in the two species. This might also suggest the existence of a major gene responsible for red skin colouration in pear in addition to minor genes. A further study in Asian pear found a single dominant gene responsible for red skin colouration and mapped the responsible gene, *R*, on LG5 (Xue et al. 2017). A study in peach reported a QTL for blush skin on LG4 (Cantín et al. 2010), explaining 68.7% of the

phenotypic variance. A further study by Frett et al. (2014) detected two minor QTLs located close to each other on LG4 in peach, one explaining ~13% of the phenotypic variance and the other explaining 13.52%. The three QTLs identified in these two studies (and located on the same linkage group) could be under the same genetic control. *Prunus* LG4 has been reported to be homoeologous with the telomeric region of LG5 in *Malus pumila* (Dirlewanger et al. 2004; Illa et al. 2011). Therefore, the QTL identified on LG5 in pear may be homoeologous to one or more of the QTLs identified on LG4 in peach.

In 'Abate Fetel', a QTL for blush was detected near the telomeric region on LG9, which varied between the years. The presence and absence of this QTL on LG9 between seasons indicate that the potential regulatory genes controlling this QTL are very responsive to environmental conditions, consistent with the reports that environmental factors, such as light and temperature, influence the development of blush colour in pear cultivars such as 'Rosemarie' (Steyn et al. 2004a, 2005; Wand et al. 2005). The presence of a major QTL in the non-blush cultivar 'Abate Fetel' could hint at the suppression role of this QTL of the genes responsible for blush skin in some seedlings and indicates that the genetic control of blush skin in pear is complex.

Candidate genes associated with blush skin development in pear

Of the three candidate *MYB86* genes identified on LG5 in apple, MDP0000289671 was located closest to the QTL region at 1464 kbp. A recent study in strawberry reported that another *MYB86* gene was upregulated in the red skin of the cultivar 'Ruegen' and downregulated in the yellow skin of 'Yellow Wonder' (Zhang et al. 2015). However, the study stated that the role of the *MYB86* gene in flavonoid biosynthesis is unknown. Of the three *UDP-glucosyl transferase* genes identified on LG5 in apple, MDP0000242552 was found located closest to the QTL region at 1321 kbp. A previous study in peach by Frett et al. (2014) reported that a *UDP-glycosyl transferase* gene co-located with one of the minor QTLs for blush detected on LG4, which is homoelogous to apple LG5. However, that study did not prove whether this gene played an important role in anthocyanin biosynthesis. Although these candidate genes appear to be located distant from the QTL region based on reported linkage disequilibrium (LD) in apple (400 kbp; Di Guardo et al. 2017), the association of these gene(s) with blush skin cannot be entirely excluded. Improved annotation and LD estimation of the European pear genome would be valuable to elucidate the association and role of these gene(s) with blush skin.

Interestingly, a study in Asian pear found the *PyMYB114* gene to be co-locate with the QTL associated with the red skin on LG5 (Yao et al. 2017). This gene was found upregulated in red skin pear cultivars such as 'Starkrimson' and downregulated in green skin pear cultivars such as 'Bartlett' ('William's Bon Chretien'). This gene could be the same gene responsible for blush skin colouration in 'Flamingo' located on LG5. However, this needs further studies to confirm this hypothesis, using either the apple or pear genome.

In other Rosaceae species, *MYB10* is involved with the red skin colour (Sooriyapathirana et al. 2010; Frett et al. 2014). In peach and sweet cherry, *PaMYB10* and *PprMYB10* genes were mapped on LG3 (Sooriyapathirana et al. 2010; Frett et al. 2014) which is homoeologous with LG9 in *Malus* species (Dirlewanger et al. 2004; Illa et al. 2011), where MYB genes including *MdMYB1* (Takos et al. 2006), *MdMYBA* (Ban et al. 2007) and *MdMYB10* (Espley et al. 2007) associated with red skin were mapped in apple. These genes could be the same genes controlling blush skin in 'Abate Fetel' detected in the current study. Pierantoni et al. (2010) mapped *PcMYB10* to the telomeric region in 'Abate Fetel', and it is possible that this co-locates with the major QTL detected in the current study. However, it is difficult to compare these two maps due to only one common SSR marker mapped by Pierantoni et al. (2010).

The current study found three *MYB39* and two *MYB21* candidate genes located near the QTL region on LG9 in the apple genome. Three *MYB39* genes were located in a region of 213 to 610 kbp from the QTL region and *MYB21* genes in a region of 357 to 358 kbp from the QTL region. The shorter distance of some of these genes from the QTL region (compared to LD of 400 kbp reported in apple; Di Guardo et al. 2017) suggests that these genes could be involved in blush skin colouration in pear. However, further studies are needed to investigate the role of these genes using the pear genome. Previous study in strawberry by Zhang et al. (2015) reported that the *MYB39* gene was upregulated in red skin and downregulated in yellow skin. However, whether this gene plays an important role in anthocyanin biosynthesis has not been reported to date. In *Arabidopsis thaliana*, the *MYB21* gene has been reported to play an important role in stamen filament elongation (Cheng et al. 2009; Song et al. 2011).

Although a stable QTL for blush skin was identified on LG5, and another on LG9, a substantial proportion of the variance was not explained. It is likely that other minor QTLs influencing blush development in this pear progeny were not detected due to the limited number of classes used to score the trait. Moreover, this trait could be largely influenced by environmental conditions. Including replicates of the seedlings of the progeny, and scoring the trait over various seasons, might improve detection of additional QTLs. Filling the gaps and increasing the saturation of the map with additional SSRs or other types of molecular markers such as SNPs could also improve the detecting additional QTLs.

Marker-assisted selection for blush skin

Although a number of PCR-based markers are available in Asian pear for traits such as scab resistance, russet skin, self-

incompatibility, fruit storage and harvest time (Yamamoto and Terakami 2016), similar markers are not available for European pear. Two markers on LG5, NB101a and SAmsCO865954, with alleles 139 and 462, respectively, displayed association with blush skin in the progeny and were also present in 'Flamingo' and in 'Forelle', the parental blush cultivar of 'Flamingo'. However, alleles associated with these markers were detected only in limited number of accessions mostly derived from 'Forelle' and one cultivar unrelated to 'Forelle' or 'Flamingo'. Moreover, further studies to test alleles 89 and 169 from markers NH029a and GD142 associated with the blush QTL on LG9 in 'Abate Fetel' together with the markers associated with blush on LG5 might be useful for selection of seedlings with good blush skin. Saturation of both LGs with additional markers would increase the possibility of detecting marker alleles that are more tightly linked to this trait. Although the mentioned markers are not robustly associated with the blush skin trait, they can be used as supplementary tools for MAS for blush skin and should be tested in more accessions.

QTL detection for flowering time

The major stable QTL found in the current study towards the telomeric region of LG9 in 'Flamingo' explained more than 30% of the phenotypic variance. This QTL was also detected in 'Abate Fetel' explaining 15.9% of the phenotypic variance. A study in apple reported a QTL associated with floral budbreak explaining 9.3% phenotypic variance that was located at the same telomeric region of LG9 (Celton et al. 2011), as the QTL found in the current study. In the 'Abate Fetel' map in 2014, this QTL was located between 0.0 to 9.6 cM above the markers NZmsCN943946 and GD142 in comparison with the region of 0.0 to 9.1 cm that was reported for floral budbreak by Celton et al. (2011). In European pear, a recent study (Gabay et al. 2017) also mapped a QTL associated with date of vegetative budbreak explaining 18.5–37.7% of the phenotypic variance in a similar region above marker NZmsCN943946 on LG9 in the progeny of 'Spadona' (low chill) × 'Harrow Sweet' (high chill). In a study by Van Dyk et al. (2010), a major QTL derived from low-chill cultivar 'Anna' and associated with date of initial vegetative budbreak was also mapped to the telomeric region of LG9 explaining 4.8–44.6% of the phenotypic variance. This QTL was previously reported in the apple cultivar 'Anna' by Hauagge and Cummins (1991) as the major dominant gene responsible for low chilling requirements in this cultivar. A more recent study in apple reported QTLs associated with flowering time and budbreak on LG9 explaining between 11.5 and 18.2% of the phenotypic variance (Allard et al. 2016). The study reported candidate genes, *AGL24* and *FLC-like*, associated with the onset of flowering at the telomeric region of LG9, and which could be possible candidate genes for the QTL identified for flowering time in the current study. The similar positions of the apple and pear QTLs suggest that they are under the same genetic control and need to be confirmed in further studies.

None of the QTL regions for flowering time reported on LG7, LG10 and LG17 by Liebhard et al. (2003); on LG1, LG6, LG12 and LG17 by Celton et al. (2011) or on LG8 and LG15 by Kunihisa et al. (2014) and Allard et al. (2016) in apple were detected in the current study.

In *Prunus* species, QTLs associated with the blooming date in peach (Dirlewanger et al. 2012; Romeu et al. 2014) and sweet cherry (Dirlewanger et al. 2012; Quero-García et al. 2014) were located on LG3 in both species. These QTLs could be homologous to those identified in the current study since LG3 in *Prunus* species is homologous with LG9 in pome fruit (Dirlewanger et al. 2004; Illa et al. 2011). Major QTLs controlling flowering time have been reported in two additional *Prunus* crops: on LG4 in almond (Sánchez-Pérez et al. 2007) and LG5 in apricot (Campoy et al. 2011).

In 'Flamingo', allele 161 from marker GD142 appeared to be associated with early to medium flowering in this progeny. Seedlings that reached 80% full bloom earlier than, or at the same time as both parents, often had allele 161, while 72% (scored in 2014), 70% (scored in 2015 and 68% (based on the average data set scores) of the seedlings flowering later than both parents lacked this allele, but had allele 167, also from marker GD142 in 'Flamingo'. In 'Abate Fetel', neither allele of marker GD142 showed association with flowering time in this progeny.

The statistical analysis for allele 161 showed that the type II error was less than 20% in both years; power and sensitivity were more than 80% and accuracy was more than 70%. Further testing in more individuals and related cultivars is needed to confirm the suitability of this marker for MAS of early to medium flowering selections in related breeding lines.

Conclusion

Blush skin and flowering time have not previously been mapped in the European pear. In this study, two SSR markers, SAmsCO865954 (allele 462) and NB101a (allele 139), associated with a major QTL for blush skin on LG5 in 'Flamingo'. The two markers, NH029a (allele 89) and GD142 (allele 169), appeared to be associated with another QTL for blush on LG9 in 'Abate Fetel'. The study identified the candidate genes *UDP-glucosyl transferase* and *MYB86* on LG5 and *MYB39* and *MYB21* on LG9 that may be involved in blush skin development in pear. Flowering time data sets indicated that allele 161 of marker GD142 on LG9 in 'Flamingo' may be associated with early to medium flowering time. If verified, these markers could accelate breeding efficiency for blush skin and early flowering in pear breeding programmes.

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Data archiving statement PCR multiplexes made up of pear and apple SSR primer pairs used to genotype the 'Flamingo' × 'Abate Fetel' pear progeny for mapping are provided in Table S1.

Blush skin data sets used to detect QTLs in the 'Flamingo' × 'Abate Fetel' progeny are provided in Table S2.

Flowering time data sets (Julian date of 80% full bloom) used to detect QTLs in the 'Flamingo' × 'Abate Fetel' progeny are provided in Table S3.

Information on markers not used for mapping in the progeny of 'Flamingo' × 'Abate Fetel' is provided in Table S4.

Genotypic data for the SSR markers in the pear progeny of 'Flamingo' × 'Abate Fetel' are provided in Table S5.

Position of the QTLs for blush skin detected on LG5 in 'Flamingo' and LG9 in 'Abate Fetel' in the pear progeny of 'Flamingo' × 'Abate Fetel' based on grouping of classes is provided in Fig. S1.

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