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Fine genetic mapping of the *Co* locus controlling columnar growth habit in apple

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Abstract Tree architecture is an important, complex and dynamic trait affected by diverse genetic, ontogenetic and environmental factors. 'Wijcik McIntosh', a columnar (reduced branching) sport of 'McIntosh' and a valuable genetic resource, has been used intensively in apple-breeding programs for genetic improvement of tree architecture. The columnar growth habit is primarily controlled by the dominant allele of gene *Co* (*columnar*) on linkage group-10. But the *Co* locus is not well mapped and the *Co* gene remains unknown. To precisely map the *Co* locus and to identify candidate genes of *Co*, a sequence-based approach using both peach and apple genomes was used to develop new markers linked more tightly to *Co*. Five new simple sequence repeats markers were developed (C1753-3520, C18470-25831, C6536-31519, C7223-38004 and C7629-22009). The first four markers were obtained from apple genomic sequences on chromosome-10, whereas the last (C7629-22009) was from an unanchored apple contig that contains an apple expressed sequence tag CV082943, which was identified through synteny analysis between the peach and apple genomes. Genetic mapping of these five markers in four F_1 populations of 528 genotypes and 290 diverse columnar selections/cultivars (818 genotypes in total) delimited the *Co* locus in a genetic interval with 0.37 % recombination between markers C1753-3520 and C7629-22009. Marker C18470-25831 co-segregates with *Co* in the 818 genotypes studied. The *Co* region is estimated to be 193 kb and contains 26 predicted gene in the 'Golden Delicious' genome. Among the 26 genes, three are putative LATERAL ORGAN BOUNDARIES (LOB) DOMAIN (LBD) containing transcription factor genes known of essential roles in plant lateral organ development, and are therefore considered as strong candidates of *Co*, designated *MdLBD1*, *MdLBD2*, and *MdLBD3*. Although more comprehensive studies are required to confirm the function of *MdLBD1-3*, the present work represents an important step forward to better understand the genetic and molecular control of tree architecture in apple.

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Introduction

Apple (*Malus × domestica* Borkh, family Rosaceae) is one of the most important fruit crops in the world. Based on the

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stats of the Food and Agriculture Organization (FAO), over 69.8 million metric tons of apple fruits were produced by 93 countries worldwide in 2008. Apple tree architecture, which varies greatly not only among cultivars but also within a tree along the course of growth and development, is an important complex trait affecting planting density, fruit quality, orchard management and production (Hemmat et al. 1997; Kenis and Keulemans 2007). Complex genetic, ontogenetic and environmental factors play a role in determining apple tree architecture and many quantitative trait loci (QTLs) of significant effect on tree form have been detected (Kenis and Keulemans 2007; Segura et al. 2008, 2009). Despite the tremendous variations, apple tree architecture has been categorized into four types based on its overall growth habit and fruiting type: columnar (I), spur (II), standard (III) and tip bearing (IV) (Costes et al. 2006; Lespinasse and Delort 1986; Pereira-Lorenzo et al. 2009).

The columnar growth habit in apple was initially discovered as a sport of 'McIntosh' (Lapins 1969). This mutant, called 'Wijcik McIntosh', has short internodes, a thick stem with little difference in diameter between the top and the base and a reduced plant height and branching (Lapins 1976). These characteristics of 'Wijcik McIntosh' have been viewed highly valuable in apple-breeding programs for the genetic improvement of tree architecture. One of the modern apple breeding goals is to produce apple cultivars with columnar growth habit for growing apple in high-density orchards (Kenis and Keulemans 2007; Moriya et al. 2009; Tobutt 1984). Due to its reduced branching, columnar trees require minimal pruning and training, and genetic selection of columnar form would allow adequate light penetration to fruits and leaves.

Some physiological studies have focused on understanding how the variations at the plant hormonal levels may associate with the types of tree architecture. Among the four major phytohormones examined, i.e., Auxins, ABA (abscisic acid), cytokinins and GA (gibberellins), higher levels of cytokinins, such as zeatin-like growth substances, appeared to be most consistently correlated with columnar growth habit (Looney and Lane 1984; Watanabe et al. 2004, 2008). These observations are in accordance with the in vitro comparisons of tissue tolerance to various cytokines or cytokinin-like compounds, such as BA (benzyl adenine), kinetin, 2iP (2-isopentenyladenine), TDZ (N-phenyl-N-1,2,3-thiadizol-5-ylurea). Among the three strains of 'McIntosh', 'Wijcik McIntosh' was more tolerant to high cytokinin than either 'Macspur' (intermediate tolerance), or 'McIntosh' (died) (Lane et al. 1982; Sarwar et al. 1998). Higher levels of IAA (indole-3-acetic acid) in subapical parts of shoot in early June and July and in columnar types were reported (Watanabe et al. 2004, 2008). In addition, IAA concentrations of columnar

were significantly higher in apical shoots than in lateral shoots while there was no such difference in standard (Watanabe et al. 2006). The role of ABA and GA on tree architecture was not as certain as that of cytokinins and IAA. However, the ratio of IAA/cytokinin was lower in columnar types, and IAA/ABA and GA_3/IAA were higher in tip-bearing types, suggesting the existence of combined roles of two or more plant hormones in shaping apple tree architecture (Bendokas and Stanys 2009). Although these physiological studies are helpful in explaining the strong apical dominance and apical control in columnar and provide useful information for knowing which plant hormone(s) might be more important in regulating apple-tree growth habit and fruiting types, our understanding of the underlying physiology remains unclear.

The columnar growth habit was suggested to be controlled by a single dominant gene, Co (columnar), with one or more modifiers to account for a consistent deficiency of columnar seedlings as expected from the 1 (columnar):1 (standard) ratio (Lapins 1976). The Co locus was first mapped to linkage group (LG) 10 of 'Wijcik McIntosh' using a set of random amplified polymorphic DNA (RAPD) markers (Conner et al. 1997, 1998) and an simple sequence repeats (SSR) marker SSR^{co} developed from one of these RAPDs, $OA11_{1000}$ (Hemmat et al. 1997). More recently, the columnar trait has been investigated using various columnar cultivars, e.g. 'Telamon', 'Tuscan' and 'Totem', or breeding selections derived from 'Wijcik McIntosh' (Fernandez-Fernandez et al. 2008; Kenis and Keulemans 2007; Kim et al. 2003; Moriya et al. 2009; Tian et al. 2005; Zhu et al. 2007), and the general map position of the Co locus on LG10 has been consistent despite a lack of common markers for direct comparison and alignment. However, the precise position of Co is less clear. On one hand, the Co locus was mapped to the same interval between two sequence-characterized amplified regions (SCAR) markers SCAR682 and SCAR216 in two independent reports (Moriya et al. 2009; Tian et al. 2005). On the other hand, the Co locus was placed in two presumably adjacent intervals: one between SSR markers CH02c11 and CH03d11 (Fernandez-Fernandez et al. 2008), the other between CH03d11 and Hi01a03 (Moriya et al. 2009). Nevertheless, efforts toward isolating the Co gene have been announced (Baldi et al. 2009).

Peach (Prunus persica Batsch) is another tree fruit genera in Rosaceae and the pillar growth habit in peach is the most comparable trait with columnar in apple. Peach pillar (PI), also known as columnar or broom, is controlled by a recessive allele at the br locus (Chaparro et al. 1994; Rajapakse et al. 1995; Scorza et al. 2002; Werner and Chaparro 2005) on peach LG-2 (Sajer et al. 2011; Sosinski et al. 2000). Peach chromosome-2 shows strong synteny with apple chromosomes 1, 2 and 7 (Illa et al. 2011), but

not with apple chromosome-10 where the *Co* locus resides. In combination with the contrasting gene action, i.e., dominant columnar (*Co*) versus recessive pillar (*br*), this synteny information enhances the impression that genes *Co* and *br* are different. The objectives of this study were to fine map the *Co* locus to clarify the genetic position of *Co* by combining genetic materials developed in three apple-breeding programs, and more importantly, to provide a foundation for the ultimate identification of the gene(s) underlying *Co* for better understanding the genetic and molecular control of tree architecture in tree fruits.

Materials and methods

Plant materials

A total of 818 genotypes, including 528 trees in four segregating populations and 290 columnar selections/cultivars, were used (Table 1; Online resources 1, 2). The first population (FN), consisted of 271 F_1 individuals, was derived from a cross of 'Fuji' (*coco*, standard growth habit) × NYCO7-G (*Coco*, a columnar selection from 'Wijcik McIntosh' × 'Suncrisp'). Population FN was planted in 2001 and grown on their seedling roots in Geneva, New York. The second population (TB) of 222 F_1 trees was derived from a cross of 'Telamon' (*Coco*, a columnar cultivar from 'Wijcik McIntosh' × 'Golden Delicious') × 'Braeburn' (*coco*, standard growth habit). The TB

population has been used in several studies (Davey et al. 2006; Kenis and Keulemans 2005, 2007; Kenis et al. 2008), and was created by the lab of Dr. Johan (Wannes) Keulemans, University of Leuven, Belgium. In this population, two vegetative clones per genotype were grafted on apple rootstock B.9 and planted in an orchard at Geneva, New York, in 2008. The third [Pinova (*coco*) × NYCO7-G (*Coco*), or PN] and the fourth ['Fiesta' (*coco*) × 'Totem'(*Coco*), or F × T] are small populations of only 24 and 11 F_1 trees, respectively. Both seed parents are cultivated varieties of standard tree form. 'Totem' (SA572-2), an ornamental columnar apple also known as 'Maypole 2000', is of complex interspecific origin (Fernandez-Fernandez et al. 2008). Population PN was also grown on their seedling roots, and was planted in the same orchard as population FN in 2001. The fourth population (F × T) was a subset of the population used in Fernandez-Fernandez et al. (2008) and was grown in East Malling, UK. Population F × T was not used in the initial mapping of *Co*, but assayed together with the columnar selections/cultivars described below.

The 290 columnar selections were developed by three apple breeding programs with 34 from Cornell University (CU), Geneva, New York, 121 from China Agricultural University (CAU), Beijing, China, and 135 from East Malling Research (EMR), Kent, UK. The 34 CU columnar selections (Online resource 1) include 31 genotypes from six different crosses and three named columnar cultivars: 'Irish Spire', 'Tuscan' ('Wijcik McIntosh' × 'Greensleeves') and 'Telamon'. The 121 CAU selections were

Table 1 List of F_1 populations and columnar selections/cultivars

Population/ selections	Description	Planting location	Number of trees	Columnar	Standard	Unclear	$P(\chi^2)$
FN	Fuji × NYCO7-G (**Wijcik McIntosh** × Suncrisp)	Cornell University, Geneva, NY	271	156	104	11	0.001 (10.40)
TB	Telamon (**Wijcik McIntosh** × Golden Delicious) × Braeburn	Cornell University, Geneva, NY	222	105	113	4	0.59 (0.29)
PN	Pinova × NYCO7-G (**Wijcik McIntosh** × Suncrisp)	Cornell University, Geneva, NY	24	16	7	1	0.06 (3.52)
F × T	Fiesta × **Totem**	East Malling Research, Kent, UK	11	5	6	0	0.76 (0.09)
CU[a,b]	Columnar selections	Cornell University, Geneva, NY	34	34	0	0	
CAU	Columnar selections (Fuji × **Telamon**)	China Agricultural University, Beijing, China	121	121	0	0	
EMR[a]	Columnar selections	East Malling Research, Kent, UK	135	135	0	0	
Total			818	572	230	16	

χ^2, Chi-square critical value (CV) calculated with the expected ratio 1:1; *P*, cumulative probability for $\chi^2 >$ CV

[a] Parentage of individual selections were listed in Online resources 1, 2

[b] Including three columnar cultivars

obtained from a population of more than 800 seedlings developed from a single cross of 'Fuji' × 'Telamon' made in 1995 (Zhu et al. 2007). The 135 EMR selections were derived from a large number of crosses and are diverse, but their columnar trait is all of 'Wijcik McIntosh' origin (Online resource 2). In addition, population GMAL 4595 ('Royal Gala' × PI 613988) of 188 F_1 trees, in which both parental genetic maps were developed (Wang et al. 2012), was used to map new markers to the Co region, such as marker CV082943STS, that were developed based on synteny approach using the peach genome, but could not be directly mapped in the Co mapping populations, or out of the Co region, such as markers C19509.316 and C7156.181, that were developed from apple genomic sequences on chromosome-10 supposed to be physically close to Co.

Growth habit evaluation

Growth habit was visually evaluated based on thickness of stem, number and crotch angle of lateral branches on the main axis and internode length. Compared with trees of standard growth habit, columnar trees have a thicker main stem characterized by similar diameter at the tip and the base, fewer lateral branches with narrower crotch angles, and shorter internodes (Hemmat et al. 1997; Kenis and Keulemans 2007). In the FN, TB and PN populations, trees were evaluated in 2009 and 2010 and classified into two categories: columnar and standard. Population F × T had already been evaluated (Fernandez-Fernandez et al. 2008). Columnar and standard selections were classified by the apple breeder.

Genomic DNA extraction and PCR amplification

Genomic DNA isolation, PCR and SSR analyses were conducted as described previously (Wang et al. 2012). Young leaves were collected and ground using a Tissue-Lyser II (Qiagen, Valencia, CA). Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method according to Doyle and Doyle (1987) and Cullings (1992). PCR amplification of SSR markers was conducted in a volume of 20 μl containing 50 ng of genomic DNA, 1 × PCR buffer, 0.2 mM of dNTP, 2 mM $MgCl_2$, 0.5 μM of each primer and 0.5 U of Taq DNA polymerase (Promega, Madison, WI, USA). The thermal cycling conditions include: an initial cycle of 5 min at 95 °C, 35 cycles of 30 s at 94 °C, 30 s at 55 °C, 90 s at 72 °C, and a final cycle of 5 min at 72 °C. PCR for RAPD analysis was conducted according to Hemmat et al. (1997). PCR products were analyzed on a 6.0 % non-denaturing polyacrylamide gel or a 2.0 % agarose gel. The ethidium bromide-stained gel images were photographed with a UVP DigiDoc-It Imaging System (UVP97010501, Fisher Scientific, Pittsburgh, PA) and segregating bands were recorded manually for linkage analysis.

Initial mapping of the Co locus

Eighteen published markers (nine SSRs, three SCARs and six RAPDs) were selected (Fernandez-Fernandez et al. 2008; Hemmat et al. 1997; Kim et al. 2003; Liebhard et al. 2002; Silfverberg-Dilworth et al. 2006; Tian et al. 2005), and their names and primer sequences are presented in Online resource 3. Association of the 18 markers with Co was first screened with ten columnar and ten standard segregants from population 'Fuji' × NYCO7-G, or nine apple varieties/selections that include four columnar ['Wijcik McIntosh', NYCO7-G, NYCO16-C ('Pinova' × NYCO7-G) and NYCO17-H ('Wijick McIntosh' × 'Redfree')] and five standard ('Fuji', 'Pinova', 'Gala,' PI 613988 and G.41). Markers that show strong association with Co were further applied to the entire mapping populations of 'Fuji' × NYCO7-G (FN), 'Telamon' × 'Braeburn' (TB), and 'Pinova' × NYCO7-G (PN). Genetic linkage analysis for initial mapping of Co was conducted with JoinMap 3.0 (Van Ooijen and Voorrips 2001) by pooling the marker and growth habit data from populations FN, TB and PN.

Molecular marker development and analysis

The draft sequences of the peach and apple genomes were released in April 2010 and August 2010 (Velasco et al. 2010), respectively. To take advantages of these genomic resources, a genome sequence-based marker development approach in combination with synteny analysis was used. DNA sequences of the six confirmed Co-linked markers (AU223548, SCAR682, CH03d11, EMPc105, Hi01a03 and Hi01b01) were obtained from either GenBank or HiDRAS (http://www.hidras.unimi.it/) (Online resource 3), and initially were used to search the peach genome and then the apple genome. The objective was to establish the corresponding relationships between the markers and their DNA sequences in the peach and apple genomes so that the general region of Co could be physically localized for developing new markers closer to Co.

Basic local alignment search tool (BLAST) search against the peach genome at the genome database for rosaceae (GDR) (http://www.rosaceae.org) using the five published markers (AU223548, SCAR682, CH03d11, EMPc105 and Hi01a03) revealed that there was not a single significant hit for marker Hi01a03. However, sequences with high similarities to the first four were identified in a region of 773 kb from 18,683 to 19,456 kb in Scaffold-8 (Table 2). To develop new SSR markers,

DNA sequence of 714 kb (19,286–20,000 kb) on Scaffold-8 that contains similar sequences for markers CH03d11 and EMPc105 were downloaded, and screened for SSR motifs (eight or more di-nucleotide repeats; or six or more tri-nucleotide repeats) using the web-based program Batch-Primer3 (http://probes.pw.usda.gov/batchprimer3/index.html) (You et al. 2008). To utilize the high transferability of gene-based markers, SSRs covered by predicted peach genes were identified by their genome position coordinates. These peach predicted SSR containing genes were used to BLAST search GenBank for *Malus* ESTs of highest sequence similarities. The resulting *Malus* ESTs were analyzed with BatchPrimer3 again for SSRs, leading to selection of six *Malus* ESTs or Unigenes that are presumably scattered over the *Co* region. Six primer pairs were designed (Online resource 4) and assayed with the set of nine columnar and standard varieties/selections mentioned above for mapping.

Similar BLAST searches against the apple genome at GDR indicated that the five published markers were all found in contigs of chromosome-10, as expected, but spreading over 2,926 kb (Table 2). However, the *Co* region between markers EMPc105 and Hi01a03 is estimated to be 1,354 kb (Table 2). DNA sequences of representative contigs in this broad *Co* region as well as those in an unanchored region but mapped to the *Co* region by marker CV082943STS were downloaded and analyzed for the presence of SSRs. Primer design and analysis were similar to that described above (Online resource 5).

Fine map construction and estimation of the physical size at the *Co* locus

The mapping populations and columnar selections/cultivars (818 genotypes in total) were analyzed with the two markers, SCAR682 and Hi01a03, which flank the *Co* locus (Fig. 1a) to identify informative recombinants. Recombinant plants were then genotyped with newly developed SSR markers for fine map construction around the *Co* locus. The physical size of the *Co* locus was estimated with physical distance (kb) between two markers (C1753-3520 and C7629-22009) immediately flanking *Co* in the homologous genomic region of 'Golden Delicious' available at GDR or The Istituto Agrario San Michele all'Adige (http://genomics.research.iasma.it/index.html).

Gene prediction and phylogenetic analysis

Gene prediction in the *Co* homologous region of 'Golden Delicious' was virtually conducted by Velasco et al. (2010). All genes predicted in the region, including those listed in the consensus gene set and those predicted by FGENESH *Arabidopsis*, were downloaded from GDR for their CDS (coding sequences) and deduced protein sequences. Selection of the *Co* candidate genes was solely based on their putative functions in the predicted gene list. Phylogenetic analysis of the deduced protein sequences of the *Co* candidate genes was performed using the MEGA4 program (Tamura et al. 2007).

Results

Segregation of growth habit

In population 'Fuji' × NYCO7-G (FN, 271 individuals), 156 and 104 trees were ranked as columnar (C) and standard (S) growth habit, respectively, and 11 trees were undetermined (Table 1). The growth habit segregation in this population was significantly distorted from the expected 1 (C):1 (S) ratio [$P_{(df=1, \chi^2 > 10.40)} = 0.001$] due to excessive columnars. Of the 222 genotypes in population 'Telamon' × 'Braeburn' (TB), 105 were classified as columnar, 113 standard, and four were not determined. Chi-square test indicated that the growth habit segregation fits the expected ratio 1: 1 [$P_{(df=1, \chi^2 > 0.29)} = 0.59$]. Growth habit segregation in the small population 'Pinova' × NYCO7-G (PN) also fits the expected 1 (16 C):1 (7 S) ratio although the *P* value is low [$P_{(df=1, \chi^2 > 3.52)} = 0.06$] (Table 1). There was one genotype with ambiguous growth habit in population PN.

Table 2 Genomic sequence positions of the published *Co*-linked markers

| Marker	Apple contig	Apple genome coordinates (contig)	Peach genome coordinates (marker)
AU223548	MDC011443.241	Chr10: 16,985,072–17,006,359	Scafold-8: 18,682,861–18,685,020
SCAR682	MDC011771.458	Chr10: 17,535,637–17,544,447	Scafold-8: 19,021,026–19,021,070
CH03d11	MDC015757.243	Chr10: 18,197,057–18,229,728	Scafold-8: 19,286,305–19,286,566
EMPc105	MDC035953.13	Chr10: 18,556,885–18,580,918	Scafold-8: 19,453,116–19,456,249
Hi01a03/Hi01b01	MDC001120.265	Chr10: 19,893,418–19,911,277	
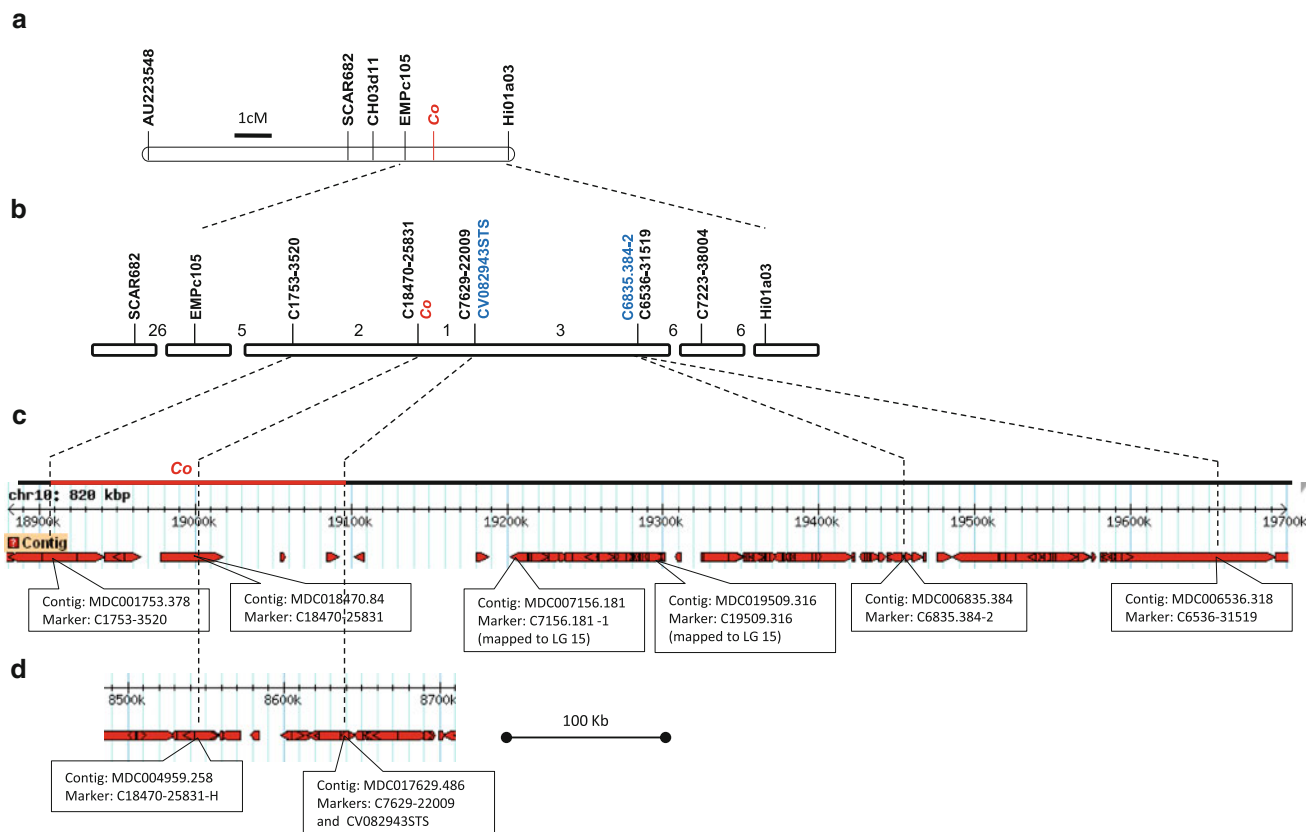
Fig. 1 Fine-scale genetic map of the *Co* locus on apple LG-10. **a** Initial map. Genetic distance (cM) between two adjacent markers is in accordance with the scale. **b** Fine-scale genetic map. The *numbers between markers* represent the number of recombinants identified out of 818 genotypes in the associated intervals. The genetic positions of markers CV082943STS (*blue*) and C6835.384-2 (*blue*) were deduced by their sequence physical locations relative to markers C7629-22009 or C6536-31519. **c** A 820 kb genome segment that spans over markers C1753-3520 and C6536-31519 on chromosome-10 of 'Golden Delicious' (a *snapshot* of a screen from the Genome Brower (GB) at http://genomics.research.iasma.it/index.html (**d** was obtained from the same GB). Markers and associated source contigs are annotated. There is a large gap of nearly 200 kb forward from marker C18470-25831. The maximum *Co* region is shown with a *red solid line*. A segment of around 100 kb, which includes two markers C7156.181-1 and C19509.316 that were mapped to LG-15 of PI 613988 in population GMAL 4595, appears to be assembled incorrectly here. **d** A 220 kb genome segment unanchored in the 'Golden Delicious' genome that spans over markers C18470-25831-H (an allele of C18470-25831) and C7629-22009 (color figure online)

Initial mapping of the *Co* locus

Screening of the 18 published markers (Online resource 3) with the ten columnar and ten standard segregants from population 'Fuji' × NYCO7-G, and/or the nine columnar and standard apple varieties/selections identified 6 markers that were strongly associated with *Co*: AU223548, SCAR682, CH03d11, EMPc105, Hi01a03 and Hi01b01. The last two markers produced nearly identical banding patterns (not shown). Closer examination showed that their primer sequences mostly overlapped, suggesting that they might represent the same marker. Therefore, only Hi01a03 was used in subsequent analyses, reducing the number of markers from six to five. Genotyping of these five markers in the three mapping populations showed that their segregation patterns were highly similar to that of columnar growth habit, i.e., fitting the expected 1:1 ratio in populations 'Telamon' × 'Braeburn' (TB) and 'Pinova' × NYCO7-G (PN)

while distorted in 'Fuji' × NYCO7-G (FN, data not shown), implicating that the growth habit segregation distortion in population FN was caused by the segregation distortion of the *Co* region. Linkage analyses were conducted using data pooled from the three populations (FN, TB and PN) of 518 individuals in total. However, the 16 genotypes of undetermined growth habit (Table 1) in the pooled dataset of *Co* were treated with a missing value in the analysis. The results demonstrated that markers EMPc105 and Hi01a03 flank the *Co* locus by 0.8 and 2.1 cM, respectively (Fig. 1a; Online resource 3).

Molecular marker development and analysis

Marker development based on the peach genome: screening of the nine columnar and standard apple cultivars/ selections with the six *Malus* EST or Unigene derived SSR/ STS (sequence tagged sites) primers (Online resource 4)

found no columnar specific bands, suggesting this approach to develop markers in the *Co* region was not effective. One reason could be that the two apple ESTs (CN912694 and CN887623) tested were not the best matches for the two intended peach genes ppa019591m and ppa011712m (Online resource 4). However, the PCR amplicons generated by the STS primers of CV082943 (Table 3; Online resource 4) was polymorphic between 'Gala' and PI 613988, the two parents of population GMAL 4595 (Fig. 2). Analyzing the STS marker with population GMAL 4595 mapped CV082943STS between markers CH03d11 and Hi01a03 on LG-10 of PI 613988, suggesting that CV082943 represents an EST from the *Co* region. Marker Hi01a03 was not present in the published version of the PI 613988 map (Wang et al. 2012), but was added in this study. BLAST searches of the apple genome with the CV082943 sequence revealed that it is nearly identical to a

part of a predicted gene MDP0000306656 in contig MDC017629.486 that has not been anchored (Table 3; Online resource 4). This suggests that contig MDC 017629.486 originates from the *Co* region on chromosome-10 and the synteny approach resulted in useful information for revising the current genome assembly around the *Co* locus.

Marker development based on the apple genome: representative contig sequences that cover most of the 1,354 kb *Co* region (with gaps) between markers EMPc105 and Hi01a03 on chromosome-10 were downloaded from GDR. Sequences in the some 160 kb region surrounding marker CV082943STS in the unanchored category of the 'Golden Delicious' genome were also similarly downloaded. Screening of these contigs using BatchPrimer3 identified a total of 36 contigs harboring at least one SSR, including 29 from chromosome-10 and 7 from the

Table 3 Name, primer sequence, fragment size and source contig of new markers developed around the *Co* locus

| Primer name	Primer sequence (for./rev.)	Primer type	Source contig	Apple genome coordinates	Prod size (bp)	SSR
C1753-3520	TCCCTCTCTTCTGTTGCAATTT	SSR	MDC01753.378	Chr10: 18,901,668–18,941,510	176	$(AT)_{21}$
	GGCTTAAAAGGGTTGAAGTTCTC					
C18470-25831	ACTCAAGGACTCTTGCCTGGT	SSR	MDC018470.84	Chr10: 18,977,912–19,017,913	169	$(TC)_{22}$
	CAACTCAATCTGCCCCAACTA					
C7629-22009	GGGCTAGCTGGCTAGGTGTA	SSR	MDC017629.486	Unanchored: 7,665,498–7,688,729	184	$(AG)_{22}$
	ACAACACACCCAGCCCTAAT					
CV082943STS[a]	GCCATGCTCCTTCCATAAAA	STS	MDC017629.486	Unanchored: 7,665,498–7,688,729	1479	
	AGGCCCACAGGATTTTTCTT					
C6835.384-2[b]	AATTGAGCAAATACTCAAAGCATGT	SSR	MDC006835.384	Chr10: 19,455,177–19,461,701	205	$(AT)_{13}$
	GGATTGAGACGCATGGGTGA					
C6536-31519	TGGATGGTGGGATTTCTTGT	SSR	MDC006536.318	Chr10: 19,590,008–19,693,186	196	$(AG)_{19}$
	AAGGAAACCACATTGCCAAA					
C7223-38004	TCCACAACTAATCCCCATTTTT	SSR	MDC007223.300	Chr10: 19,740,828–19,807,025	189	$(TA)_{18}$
	AGCATGCATGGTCTTTACCC					
C7156.181-1[c]	GACATTCCAAGATGTTCGGACAAA	SSR	MDC007156.181	Chr10: 19,202,017–19,213,346	202	$(AT)_{14}$
	TCGAGTTGGTCACTTCCCATGA					
C19509.316[d]	GGTGTTTTGTTCCCTTTTTAATTCC	SSR	MDC019509.316	Chr10: 19,290,413–19,301,048	201	$(AT)_{17}$
	CAATGAGGCTACGGCCCAAC					

[a] Mapped to LG-10 of PI 613988 (Wang et al. 2012). The reverse primer (AGGCCCACAGGATTTTTCTT) is directly derived from EST CV082943, but the forward primer (GCCATGCTCCTTCCATAAAA) is from UGID: 1640882 encompassing EST CV082943

[b] Mapped to LG-10 of PI 613988

[c] C7156.181 linked with marker C3740 on LG-15 of PI 613988

[d] C19509.316 linked with marker C4299 on LG15 of PI 613988

Fig. 2 Agarose gel (2.0 %) profiles of marker CV082943STS. *Lanes 1–9*: CV082943STS PCR products amplified from nine apple cultivars/selections of columnar or standard growth habit (shown). *Lane 10*: 1 kb Plus DNA Ladder (Invitrogen, CA). *Lane 11–25*: CV082943STS PCR amplicons from the two parents and 13 progeny of population GMAL 4595 (Wang et al. 2012)
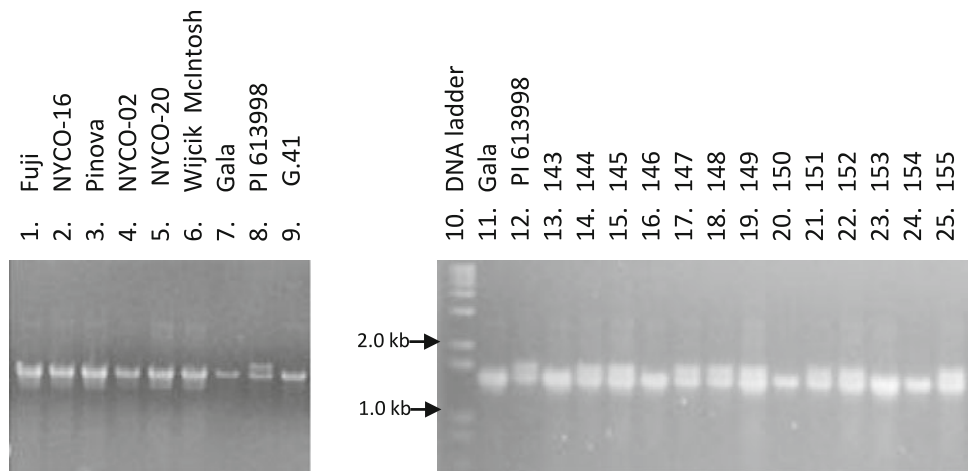
unanchored contigs, for which 67 and 21 SSR primer pairs (88 in total) were designed, respectively (Online resource 5). Evaluation of these SSR primers with the columnar/standard cultivars, selections and segregants showed that five (C1753-3520, C18470-25831, C7629-22009, C6536-31519 and C7223-38004) generated columnar-specific bands (Fig. 3) although the majority was monomorphic. Fine mapping of these five SSR markers confirmed that their close linkage to *Co* (Fig. 1b). Marker C7629-22009 is derived from the unanchored contig MDC017629.486, in which marker CV082943STS resides.

Markers C6835.384-2, C7156.181-1 and C19509.316 produced no columnar-specific bands in the three mapping populations, but they were mapped in population GMAL 4595, similar to marker CV082943STS. As expected, marker C6835.384-2 closely linked to marker CV082943STS on LG-10 of PI 613988 (Table 3; Fig. 1c; Online resource 5), However, markers C7156.181-1 and C19509.316 were unexpectedly mapped to LG-15 of PI 613988 (Table 3; Fig. 1c; Online resources 5), with marker C7156.181-1 closely linked to marker C3740, and marker C19509.316 to marker C4299 (Wang et al. 2012), suggesting that contigs MDC007156.181 and MDC019509.316 (Online resource 6), from which the two markers were derived, were likely incorrectly assembled to chromosome-10 in the apple genome sequencing project (Velasco et al. 2010).

Construction of the fine map and estimation of the physical size at the *Co* locus

Based on the initial map of *Co* (Fig. 1a), two markers (SCAR682 and Hi01a03) were used to genotype the 290 columnar selections as well as the F × T population of 11 trees (Table 1). Together with the genotypic data obtained from the three populations of 517 trees (Table 1) in the initial mapping process, 47 informative recombinants were

identified between the markers SCAR682 and Hi01a03. Of these recombinants, 10 (3.7 %) and 9 (4.1 %) were identified from populations FN and TB, respectively, and 1 (2.9 %), 23 (17.0 %) and 4 (3.3 %) from the columnar selections CU, EMR and CAU, respectively. Overall, 20
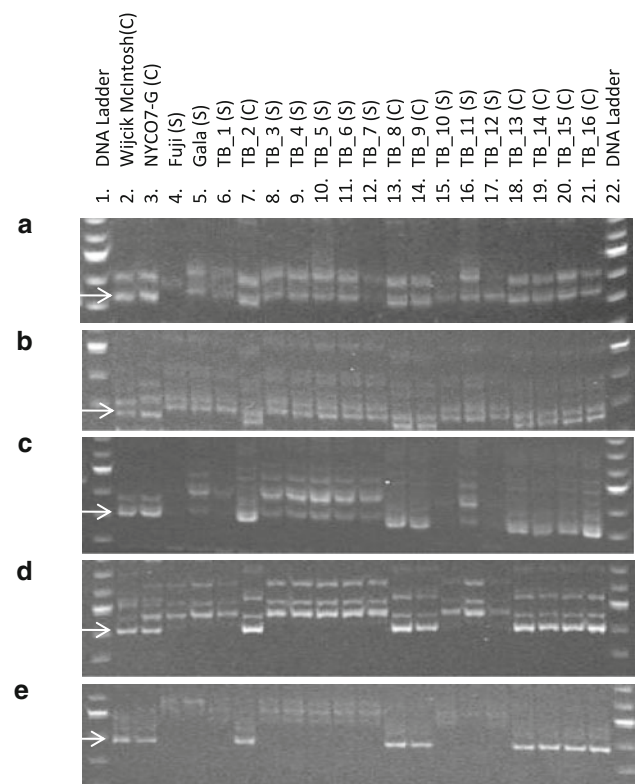
Fig. 3 Polyacrylamide gel profile of five SSR markers developed in the *Co* region. **a** C1753-3250. **b** C18470-25831. **c** C7629-22009. **d** C6536-31519. **e** C7223-38004. *Lanes 1 and 22*: 20 bp EZ Load Molecular Ruler (BioRad Cat # 170-8201, Hercules, CA). *Lanes 2–5*: four columnar and standard apple varieties/selections as shown. *Lanes 6–21*: 16 progeny from population 'Telamon' × 'Braeburn'. Bands characteristic to a specific SSR marker are indicated with an *arrow*. *C* columnar. *S* standard

recombinants arose from a *C* allele (in coupling with allele *Co*) at marker SCAR682 recombined with a *c* (in coupling with allele *co*) allele at marker Hi01a03, and 27 from a *c* allele at marker SCAR682 with a *C* allele at marker Hi01a03. There was one double-recombinant (CAUC10) between the two markers from the CAU columnar selections (Fig. 4).

Genotyping of the 47 single- and one double-recombinant(s) with the five new SSR markers (C1753-3520, C18470-25831, C7629-22009, C6536-31519, C7223-38004) allowed the identification of six most informative recombinants (Fig. 4), enabling construction of a fine haploid map at the *Co* locus (Fig. 1b). Based on this map, the *Co* locus was sandwiched by two flanking markers C1753-3520 (supported by two key recombinants EMRC-5 and EMRC-73) and C7629-22009 (supported by one key recombinant FN11-203) in an interval with a recombination frequency of 0.37 % (3/818), and marker C18470-25831 co-segregates with *Co*.

The physical size of the *Co* locus defined by the two immediate flanking markers C1753-3520 and C7629-22009 was estimated to be 193 kb (Fig. 1c, d; Online resource 6). The estimate was obtained from the sum of two genomic segments: one is from chromosome-10 of 98 kb between markers C1753-3520 and C18470-25831; the other from an unanchored segment of 95 kb between markers C7629-22009 and C18470-25831-H (a homologous sequence of marker C18470-25831, see below). The 95 kb unanchored segment includes contig MDC017629.486 on one end and contig MDC004959.258 on the other. Contig MDC017629.486 was already mapped to the *Co* region based on markers C7629-22009 and CV082943STS. Contig MDC004959.258 was identified in the process intended to fill the large gap forward from contig MDC018470.84 (Fig. 1c) in the *Co* region. Evidence

supporting contig MDC004959.258 of the origin at the *Co* locus includes the following. (1) Contig MDC004959.258 contains a sequence of 141 bp highly identical to the SSR flanking sequences of marker C18470-25831 (169 bp in Golden Delicious), i.e., 68/73 and 38/41, respectively, suggesting that a homologous sequence of marker C18470-25831, C18470-25831-H, is likely an allele of marker C18470-25831 (Online resource 7). (2) Contig MDC004959.258 (26.1 kb) shares high sequence identity with contig MDC018470.84 (40.0 kb), suggesting that MDC004959.258 may represent a homologous contig of MDC018470.84 (Online resource 7). (3) Contig MDC004959.258 is unanchored and located closely with the mapped contig MDC017629.486 (about 70 kb apart, Fig. 1d; Online resource 6).

Overall, the evidence also strongly suggests that the entire unanchored region between the two contigs MDC004959.258 and MDC017629.486 is originated from the *Co* region. The genomic segment between markers C18470-25831-H and C7629-22009 was measured at 95 kb (Online resource 6), supporting the estimated *Co* region (between markers C1753-3520 and C7629-22009) of 193 kb in 'Golden Delicious' (Online resource 6).

Predicted genes in the *Co* region

In the *Co* region of an estimate of 193 kb between markers C1753-3520 and C7629-22009, there are 26 genes predicted, including 12 from chromosome-10 (in consensus gene set) and 14 from the unanchored (include those in the consensus gene set and those predicted by FGENESH *Arabidopsis*) although there are several large gaps (Online resources 6 and 8). Based on the genome annotation (Velasco et al. 2010), the 26 genes comprise four 2OG-Fe(II) oxygenases, three LATERAL ORGAN BOUNDARIES
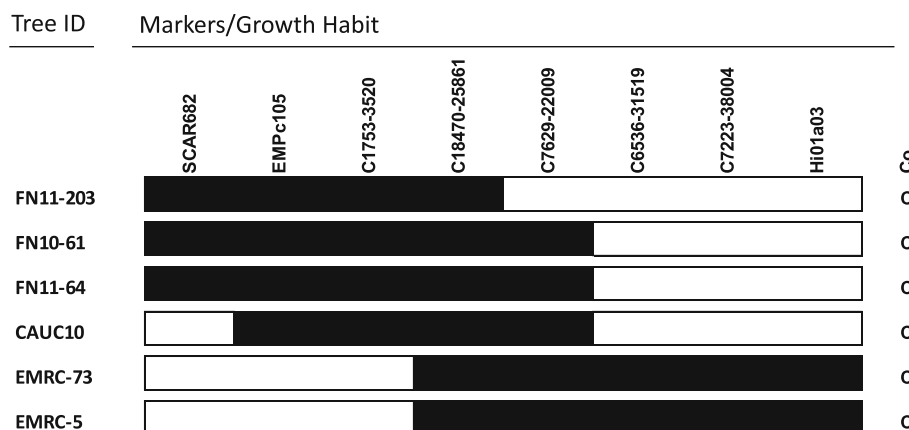
Fig. 4 Diagram of key recombinants in the *Co* region. The *solid bars* represent chromosomal segments linked in coupling phase to the *Co* allele conferring columnar growth habit from 'Wijcik McIntosh'. The *open bars* are for chromosomal segments associated with standard growth habit. The crossover break-points are shown by the junctions between the *solid and open bars*. Markers are positioned equidistantly irrespective to their genetic distance

(LOB) DOMAIN (LBD) transcription factors, three pentatricopeptide repeat proteins, two carboxylesterases, two zinc ion binding proteins, one protein serine/threonine kinase, one hydrolase, six proteins without descriptions and four others (Online resources 6 and 8) (Velasco et al. 2010). The three putative LBD transcription factors, designated MdLBD1 (MDP0000174086), MdLBD2 (MDP 0000130894) and MdLBD3 (MDP0000131964), appear to be strong candidates for CO as studies in *Arabidopsis* and other plant species indicated that LBD proteins play important roles in the development of leaf, flower and roots, in meristem programming and in embryonic and vascular patterning (Majer and Hochholdinger 2011).

To understand the phylogenetic relationship with other known LBDs, the deduced protein sequences of MdLBD1-3 were analyzed together with the 42 LBD proteins encoded by the *Arabidopsis* genome (Matsumura et al. 2009). The results categorized the three MdLBDs into Class I Subtype C (Fig. 5). MdLBD1 and MdLBD2 are most
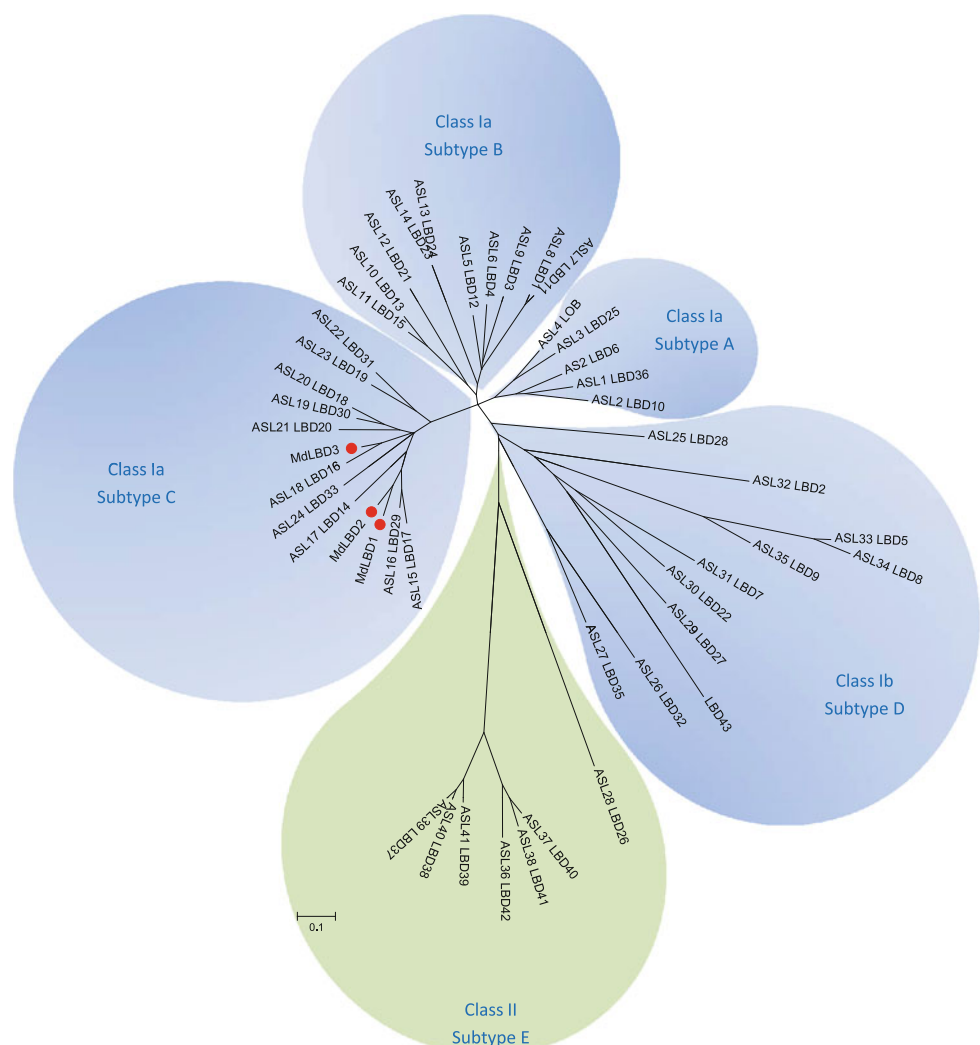
closely related to ASL16/LBD29 (At3g58190), whereas MdLBD3 most to ASL18/LBD16 (At2g42430) (Figs. 5, 6).

Discussion

Plant materials and growth-habit evaluation

The genetic materials used in this study are diverse not only in their genetic background but also in the geographic locations where these materials were developed. To minimize the possible bias in evaluating growth habit, we choose to use only columnar selections from locations outside Geneva, New York, where both segregation populations and columnar selections were grown and evaluated. As these selections are selected by breeders for columnar apple development, their columnar growth habit is considered typical. Three segregation populations were evaluated over two consecutive years in Geneva, NY. A few intermediate types were difficult to determine and were

Fig. 5 Phylogenetic analysis of the three MdLBD proteins, candidates of CO. Using the MEGA4 program (Tamura et al. 2007), the analysis was conducted on 46 LBD proteins. They include MdLBD1 (MDP0000174086), MdLBD2 (MDP0000130894), MdLBD3 (MDP0000131964), and 43 members from the LBD family of transcription factors in Arabidopsis (TAIR10), one (AT5G15060, tentatively named LBD43) more than the 42 members described in Matsumura et al. (2009). Their system used in classifying the LBD proteins (Matsumura et al. 2009) is adapted as presented. The tree is drawn to scale and the evolutionary distances are in the units of the number of amino acid substitutions per site
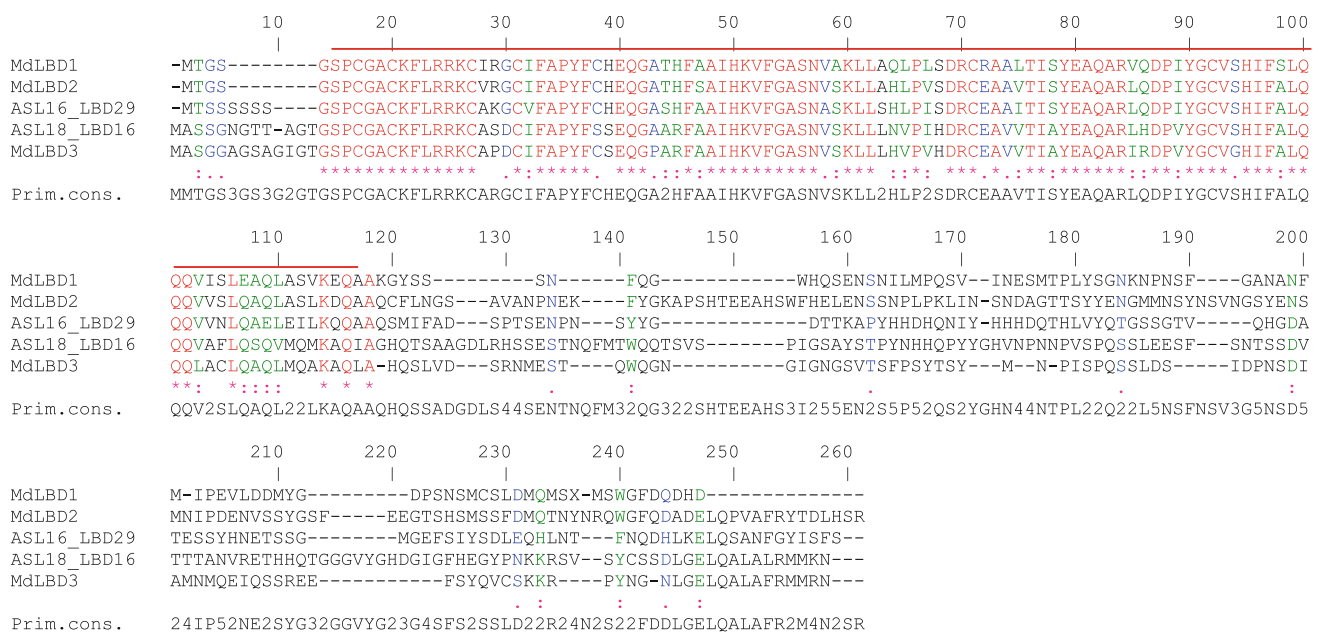
```
                 10        20        30        40        50        60        70        80        90       100
                  |         |         |         |         |         |         |         |         |         |
MdLBD1      -MTGS--------GSPCGACKFLRRKCIRGCIFAPYFCHEQGATHFAAIHKVFGASNVAKLLAQLPLSDRCRAALTISYEAQARVQDPIYGCVSHIFSLQ
MdLBD2      -MTGS--------GSPCGACKFLRRKCVRGCIFAPYFCHEQGASHFSAIHKVFGASNVSKLLAHLPVSDRCEAAVTISYEAQARLQDPIYGCVSHIFALQ
ASL16_LBD29 -MTSSSSS----GSPCGACKFLRRKCAKGCVFAPYFCHEQGASHFAAIHKVFGASNASKLLSHLPISDRCEAAITISYEAQARLQDPIYGCVSHIFALQ
ASL18_LBD16 MASSGNGTT-AGTGSPCGACKFLRRKCASDCIFAPYFSSEQGAARFAAIHKVFGASNVSKLLLNVPIHDRCEAVVTIAYEAQARLHDPVYGCVGHIFALQ
MdLBD3      MASGGAGSAGIGTGSPCGACKFLRRKCAPDCIFAPYFCSEQGPARFAAIHKVFGASNVSKLLLHVPVHDRCEAVVTIAYEAQARIRDPVYGCVGHIFALQ
             :.:.       *************  .*:*****. ***.::*:*********** .*** ::*:. ***.*.:**:*****::**:****.***:**
Prim.cons.  MMTGS3GS3G2GTGSPCGACKFLRRKCARGCIFAPYFCHEQGA2HFAAIHKVFGASNVSKLL2HLP2SDRCEAAVTISYEAQARLQDPIYGCVSHIFALQ

                110       120       130       140       150       160       170       180       190       200
                  |         |         |         |         |         |         |         |         |         |
MdLBD1      QQVISLEAQLASVKEQAAKGYSS---------SN------FQG------------WHQSENSNILMPQSV--INESMTPLYSGNKNPNSF----GANANF
MdLBD2      QQVVSLQAQLASLKDQAAQCFLNGS---AVANPNEK----FYGKAPSHTEEAHSWFHELENSSNPLPKLIN-SNDAGTTSYYENGMMNSYNSVNGSYENS
ASL16_LBD29 QQVVNLQAELEILKQQAAQSMIFAD---SPTSENPN---SYYG------------DTTKAPYHHDHQNIY-HHHDQTHLVYQTGSSGTV-----QHGDA
ASL18_LBD16 QQVAFLQSQVMQMKAQIAGHQTSAAGDLRHSSESTNQFMTWQQTSVS-------PIGSAYSTPYNHHQPYYGHVNPNNPVSPQSSLEESF---SNTSSDV
MdLBD3      QQLACLQAQLMQAKAQLA-HQSLVD---SRNMEST----QWQGN----------GIGNGSVTSFPSYTSY---M--N-PISPQSSLDS-----IDPNSDI
             **:   *::::   * * *                                  .                                   .
Prim.cons.  QQV2SLQAQL22LKAQAAQHQSSADGDLS44SENTNQFM32QG322SHTEEAHS3I255EN2S5P52QS2YGHN44NTPL22Q22L5NSFNSV3G5NSD5

                210       220       230       240       250       260
                  |         |         |         |         |         |
MdLBD1      M-IPEVLDDMYG---------DPSNSMCSLDMQMSX-MSWGFDQDHD--------------
MdLBD2      MNIPDENVSSYGSF-----EEGTSHSMSSFDMQTNYNRQWGFQDADELQPVAFRYTDLHSR
ASL16_LBD29 TESSYHNETSSG--------MGEFSIYSDLEQHLNT---FNQDHLKELQSANFGYISFS--
ASL18_LBD16 TTTANVRETHHQTGGGVYGHDGIGFHEGYPNKKRSV--SYCSSDLGELQALALRMMKN---
MdLBD3      AMNMQEIQSSREE----------FSYQVCSKKR----PYNG-NLGELQALAFRMMRN---
             . :         : :      .  :
Prim.cons.  24IP52NE2SYG32GGVYG23G4SFS2SSLD22R24N2S22FDDLGELQALAFR2M4N2SR
```

Fig. 6 Alignment of the deduced protein sequences of the three MdLBDs with two closest *Arabidopsis* LBDs (LBD16 and 29). The LOB domains are *overlined* as indicated in Matsumura et al. (2009)

removed from linkage analysis, but growth habit for most plants were scored with confidence.

Map position of the *Co* locus

In our initial mapping of the *Co* locus, five published markers (AU223548, SCAR682, CH03d11, EMPc105, and Hi01a03) generated polymorphic bands linked to the *Co* allele in coupling phase, allowing construction of an initial *Co* allele specific map (Fig. 1a). This map placed *Co* in an interval between markers EMPc105 and Hi01a03. Considering the previously published maps (Conner et al. 1997, 1998; Fernandez-Fernandez et al. 2008; Kenis and Keulemans 2007; Kim et al. 2003; Moriya et al. 2009; Tian et al. 2005; Zhu et al. 2007), the initial *Co* map is most comparable to the maps described by Moriya et al. (2009) based on the co-linearity of four common markers (AU223548, SCAR682, CH03d11 and Hi01a03). However, marker EMPc105 was not present in Moriya et al. (2009), but the relative marker positions between CH03d11 and EMPc105 were consistent with the map in Fernandez-Fernandez et al. (2008) although they mapped *Co* on the other side of marker CH03d11. Based on the two common markers (CH03d11 and SCAR682), the initial map position of the *Co* locus established in this study also appeared to be in agreement with that in Tian et al. (2005), which is more comparable with the maps in Moriya et al. (2009) according to the colinearity of three common markers (CH03d11, SCAR682 and SCAR216). The *Co* maps developed in other studies (Conner et al. 1997, 1998; Kenis

and Keulemans 2007; Kim et al. 2003; Zhu et al. 2007) could not be compared due to the lack of common markers.

Marker development and fine mapping

We developed seven new SSR/STS markers that mapped to the expected *Co* region directly (for markers C1753-3520, C18470-25831, C7629-22009, C6536-31519 and C7223-38004) or indirectly (for markers CV082943STS and C6835.384-2) using the genome sequence information of apple and peach (Table 3; Fig. 1b–d; Online resources 4–6). Apple (sub-family Maloideae) and peach (sub-family Prunoideae) are genera in the Rosaceae family. Compared with apple, peach has a relative small diploid genome and the current version of peach genome (v1.0) has more complete coverage than the apple genome (v1.0). The synteny approach utilizing the peach genome led to the development of marker CV082943STS that was essential for the construction of the *Co* fine map. Marker CV082943STS enabled development of a key marker C7629-22009 flanking *Co* and provided evidence to prove an unanchored contig (MDC017629.486) that is, in fact, of the origin at the *Co* locus in apple. In addition, our sequence-based approach developed two markers (C7156.181-1 and C19509.316) that were mapped to LG-15 of PI 613988 (Wang et al. 2012), despite the source sequences were assembled to a physical position supposed to be close to or within the *Co* region on chromosome-10 (Fig. 1c; Online resources 5). This unexpected development of the two markers might have helped correct an assembly error in the apple genome.

The fine map was constructed by analyzing the five new SSR markers on 47 single- and one double-recombinant plants, among which six were key in ordering these markers (Fig. 4). Key recombinants FN11-203, FN10-61 and FN11-64 are columnar segregants from population 'Fuji' × NYCO7-G, and CAUC10 is a double-recombinant and a columnar selection from 'Fuji' × Telamon (Table 1; Fig. 4). The pedigree of the remaining two, EMRC-5 and EMRC-73 that were developed by EMR (Fig. 4; Online resource 2), is complex: EMRC-5 is a columnar selection from the cross 'Red Sentinel' × **SA723-17** ['**Maypole**' ('**Wijcik McIntosh**' × *Malus* 'Baskatong') × 'Dorothea'], whereas EMRC-73 from a hybridization of **SA320-3** [**SA54-290** (A1583 × '**Wijcik McIntosh**') × 3762] × AR633-1, where the apple cultivars/selections in bold are columnar. This pedigree information suggested that the six key recombinants all probably inherited their columnar growth habit from 'Wijcik McIntosh'.

Recombinants FN11-203, EMRC-5 and EMRC-73 are the most informative in delimiting the *Co* region to the shortest interval between markers C1753-3520 and C7629-22009. Based on the deduced physical size of 193 kb (Fig. 1c, d; Online resource 6) for this genetic interval in the 'Golden Delicious' genome, the map restricted *Co* to a manageable genomic segment to begin searching for the candidate genes of *Co*. Our approach using diverse plant materials appeared to be a positive aspect in construction of the fine map as many more genotypes might have to be screened to narrow down the *Co* region to 193 kb. Although we defined the *Ma* locus controlling apple fruit acidity to a 150 kb region using only 438 trees (Xu et al. 2011), the *Co* map developed from 818 genotypes is highly compared with the apple *Vf* fine map of 350 kb that required the analysis of 2,071 trees from seven F_1 populations (Patocchi et al. 1999; Vinatzer et al. 2001), and the rice *Sub1* fine map of 150 kb that was developed from more than 4,000 F_2 plants (Xu et al. 2000, 2006). The high recombination frequency (17.0 %) in the EMR columnar selections, which was derived from a set of diverse parental lines (Online resource 2), was vital for the successful fine-scale mapping at the *Co* locus. One possible explanation for such high recombination rate in the EMR selections could be that some of the parents used in the pedigree might tend to generate gametes of higher crossover rate at the *Co* locus. This appeared to be the case at the tomato *Mi* locus conferring root-knot nematode resistance where the recombination frequency varied by eightfold among different populations studied (Kaloshian et al. 1998).

Estimation of the physical size of the *Co* locus

The fine map defines the *Co* locus between two markers C1753-3520 and C7629-22009 with the former derived from contig MDC001753.378 on chromosome-10 and the latter from contig MDC017629.486 that is unanchored in the draft genome sequence of 'Golden Delicious' (Velasco et al. 2010). The unanchored contig MDC017629.486 encompasses CV082943, an apple EST orthologous to a peach gene ppa000811m found in a genomic region in Scaffold-8 presumably orthologous to the apple *Co* region (Online resource 4). Although CV082943 could not be mapped in the three mapping populations due to lacking of polymorphism, a CV082943 derived STS marker CV082943STS was mapped between markers CH03d11 and Hi01a03 on LG-10 of *M. sieversii* PI 613988 in population GMAL 4595 (Wang et al. 2012). These data, together with the independent SSR marker C7629-22009 developed directly from contig MDC017629.486, confirm that the unanchored MDC017629.486 originated from the *Co* region.

Estimation of the physical size of 193 kb at the *Co* locus between two markers C1753-3520 and C7629-22009 was conducted by combining a genomic segment from chromosome-10 of 98 kb between markers C1753-3520 and C18470-25831 and the other from an unanchored segment of 95 kb between markers C18470-25831-H and C7629-22009. There is no doubt that the 98 kb segment on chromosome-10 is of the *Co* locus origin. Important evidence in supporting that the 95 kb unanchored segment is also of the origin of the *Co* locus includes: (1) contig MDC017629.486 encompasses markers C7629-22009 and CV082943STS; (2) contig MDC004959.258 share high sequence identity with contig MDC018470.84 and contains marker C18470-25831-H (Online resource 7); (3) contig MDC004959.258 is placed only 70 kb apart from the mapped contig MDC017629.486 (Fig. 1d).

Predicted genes in the *Co* region

There are 26 genes predicted in the *Co* region estimated of 193 kb between markers C1753-3520 and C7629-22009. Among them, the three putative LBD transcription factors, *MdLBD1-3*, appear to be strong candidates for *Co* based on the known functions of characterized LBD proteins in regulating lateral organ development in *Arabidopsis* and other plant species (Majer and Hochholdinger 2011). Phylogenetic analysis of the deduced protein sequences of MdLBD1-3, together with the 42 LBD proteins encoded by the *Arabidopsis* genome (Matsumura et al. 2009), categorizes the three MdLBDs into Class I Subtype C (Fig. 5). MdLBD1 and MdLBD2 are closely related to each other and resemble ASL16/LBD29 (At3g58190) the most while MdLBD3 is most similar to ASL18/LBD16 (At2g42430) (Figs. 5, 6). In a model of the auxin response factor (ARF)7- and ARF19-dependent auxin signaling cascade for lateral root formation, the activity of LBD16 and

LBD29 directly leads to division of pericycle cells and lateral root initiation (Okushima et al. 2007). Given the known involvement of IAA in columnar growth habit (Watanabe et al. 2004, 2006, 2008), whether or not one, two or all of the three MdLBDs participate in controlling the growth and development of apple lateral branches, an important function of CO, appears to be a promising future direction.

In summary, our research has resulted in a fine-scale genetic map at the *Co* locus. Based on this map, the *Co* locus co-segregates with marker C18470-25831 in an interval between markers C1753-3520 and C7629-22009 in the 818 genotypes studied. The five *Co* tightly linked SSR markers would be useful in marker assisted selection of columnar apples at the young seedling stage before planting. The physical size of the *Co* region defined by the two markers C1753-3520 and C7629-22009 was estimated to be 193 kb in the 'Golden Delicious' genome. There are 26 predicted genes in the *Co* region, including the three LBD transcription factor genes (*MdLBD1–3*) that putatively play an important role in regulating lateral organ development in plant, which makes *MdLBD1-3* strong candidates of *Co*. It would be of great interest to characterize the function of the *Co* candidate genes *MdLBD1–3* in future to better understand the genetic and molecular control of columnar growth habit and tree architecture in apple.

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