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Molecular-marker analysis of quantitative traits for growth and development in juvenile apple trees

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Abstract Random amplified polymorphic DNAs (RAPDs) were used in combination with a double pseudo-testcross mapping strategy to estimate the position and effects of quantitative trait loci (QTLs) for traits influencing juvenile tree growth and development in two apple cultivars. The mapping population consisted of 172 F_1 trees from a cross between the columnar mutant 'Wijcik McIntosh' and a standard form disease-resistant selection NY 75441-58. Significant associations were found between markers and height increment, internode number, internode length, base diameter increment, base diameter after 9 years of growth, branch number, and leaf break. The number of genomic regions associated with each trait varied from one to eight. The amount of variation explained by linear regression on individual marker loci (R^2) ranged from 3.9 to 24.3%, with an average of 7%. Multiple regression using markers for each putative QTL explained from 6.6 to 41.6% of the phenotypic variation, with an average value of 24.3%. A large number of traits had significant variation associated with the map position of the dominant columnar gene, *Co*. QTL stability over years was estimated by comparing the locations of putative QTLs for traits measured in multiple years. The majority of genomic regions were associated with a trait in only a single year, although regions associated with a trait in more than 1 year were also detected. The limitations of dominant markers and an outbred mapping pedigree for QTL analysis are discussed.

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Introduction

Apple (*Malus* × *domestica*) tree vigor and plant form are commonly manipulated through the use of size-controlling rootstocks, pruning, and training. Genetic modification of plant form and vigor are desirable goals in both scion and rootstock breeding, but improvement efforts have been hampered by a poor understanding of the inheritance of many morphological traits in apple.

The inheritance of apple-tree development has been difficult to study because the parental cultivars are budded or grafted onto rootstocks that influence tree vigor and architecture, while apple seedlings are usually grown on their own roots. Thus comparisons between parents and progenies and estimations of genetic parameters for seedling-vigor traits are difficult. However, the close relationship found between juvenile period and seedling diameter indicates a strong genetic component for vigor (Visser 1970). In one of the few genetic studies on apple vigor, Watkins and Spangelo (1970) found 100% additive variance for vigor in scion selections as measured by plant height after the first season.

'Wijcik McIntosh', a genetic sport affecting the plant form of 'McIntosh', offers prospects for studying genes influencing apple-tree form. This sport has a reduced number of lateral branches, an increased number of fruit spurs, and compaction of internodes (Lapins 1969). The columnar, or reduced branching, phenotype was reported to be controlled by a single dominant gene, *Co*, with one or more modifiers postulated to account for a consistent deficiency of compact

seedlings (Lapins 1976). Hemmat et al. (1997) created allele-specific primers for a RAPD linked to *Co* for use in selection, because in some populations it is difficult to differentiate columnar seedlings from those with a standard habit. 'Wijcik McIntosh' is being used as a parent to produce compact cultivars and to investigate the genetics of plant architecture.

Molecular markers associated with many simply inherited traits in apple have been identified, including V_f resistance to scab (Koller et al. 1994; Manganaris et al. 1994; Hemmat et al. 1995; Gardiner et al. 1996; Tartarini 1996; Yang and Korban 1996), *Pl1* powdery mildew resistance (Markussen et al. 1995); fruit color, R_f (Cheng et al. 1996); and columnar growth habit, *Co* (Hemmat et al. 1997). Lawson et al. (1995) searched for molecular markers linked to several developmental traits in apple and found markers and genes for lateral branching, vegetative bud break, and root suckering. However, due to the small size of the mapping population, only major gene effects were examined.

Genetic maps have been created for the cultivar 'Wijcik McIntosh' and two advanced breeding selections by analyzing two half-sib progenies with RAPD markers (Conner et al. 1997). In the present report we examine linkages between molecular markers and quantitative traits related to apple-tree morphology and development in one of these populations. These traits display a continuous phenotypic distribution and marker loci are used to estimate the number and effect of QTLs (quantitative trait loci) associated with these characters. These markers will aid apple breeding by increasing our understanding of the inheritance of these characters and the prospects for marker-assisted selection.

Materials and methods

Plant materials and measurements

A population of 172 trees from the cross 'Wijcik McIntosh' (WM) × NY 75441-58 (NY-58) was used for QTL analysis. The cross was made in 1986 by S.K.B., and the progeny was designated NY 86229. WM is a spur-type mutation of 'McIntosh' and is heterozygous for the dominant columnar *Co* gene (Lapins 1976). NY-58 lacks *Co*, has a standard plant form, and is heterozygous for the dominant V_f gene for resistance to apple scab. Seeds were planted into flats in the greenhouse in January, 1987, and screened for resistance to scab in February. Seedlings from the population were part of the scab-resistance breeding program, and were screened for V_f resistance, and susceptible plants (60%) removed. This resulted in skewed segregation ratios for several markers on LG 8 linked to V_f (Conner et al. 1997). Resistant plants were transplanted into 4-inch pots after scab screening and grown in the greenhouse until May, 1987, when they were transplanted into the field. Seedlings were grown on their own roots in orchards at the New York State Agricultural Experiment Station in Geneva, New York. Trees were grown at 91-cm spacing within the row and were not pruned. A standard commercial spray regime was used to control disease and insect pests.

Tree vigor was assessed using tree-height and base-diameter measurements (See Table 1). Measurements were expressed as the amount of growth in a single season. First-year measurements were taken in October 1987, after growth cessation. Year 2 and 3 measurements were taken in the fall of 1988 and 1989 after all growth had ceased. Base diameter was measured again in 1995 after 9 years of growth to determine correlations between early growth measurements and mature tree size. Branch numbers were counted in 1987 after the 3rd year of growth.

Leaf break and columnar form were rated in 1994. Seedlings were visually assessed as having either columnar or standard form. Leaf break was determined by scoring trees five times at weekly intervals beginning April 14. The following scale was used: (1) no development; (2) green tip on the terminal bud; (3) green tip on terminal and lateral buds; (4) small leaves present; (5) leaves 3–5 cm long; (6) leaves greater than 5-cm long. Scores from the five dates were summed to produce a measure of leaf flush, with higher scores indicating an earlier bud break.

Molecular-marker and QTL analysis

DNA isolation, RAPD and isozyme analysis, and linkage mapping were performed as described in Conner et al. (1997). Genetic maps were constructed for each parent using the double pseudo-testcross mapping strategy (Weeden 1994) and JoinMap software (Stam 1993) (see Discussion). The WM map consisted of 181 markers comprising 18 linkage groups with a total map length of 858 cM. The NY-58 map contained 183 markers arranged into 18 linkage groups covering 898 cM. Linkage distances varied from 0 to 27 cM, with an average distance of 5 cM between markers in each map. Homologous linkage groups in the individual cultivar maps were aligned through the use of doubly heterozygous markers that serve as "locus bridges" (Echt et al. 1993) between the maternal and paternal maps.

A subset of the markers from the genetic linkage maps was used for QTL analysis. Markers deviating significantly from expected Mendelian segregation ratios ($x^2 < 0.01$) and markers with fewer than 100 data points were excluded from QTL analysis. The normality of quantitative trait distributions was evaluated using histograms and normal probability plots. All statistical analyses were performed using MINITAB statistical software. Associations between markers and quantitative traits were determined using single-factor ANOVA of the trait means for the two genotypic classes at each RAPD marker locus (one-way ANOVA procedure). Significant *F*-values ($P \leqslant 0.01$) were interpreted to indicate linkage between a marker and a QTL. A total of 3029 single ANOVA tests (233 markers × 13 traits) were performed for this analysis. With a significance threshold of 0.01 for individual tests there is a great chance of making a type-I error, i.e., proclaiming a QTL where there is none. However, raising the significance threshold will increase the chance of overlooking important loci. Therefore a threshold of $P \leqslant 0.01$ was used with the understanding that putative QTLs will need to be confirmed in additional studies.

The proportion of phenotypic variation explained by individual QTLs was estimated by simple linear regression between the marker and the trait. When more than one marker from a single linkage group was linked to a QTL, the R^2 values generally peaked at one marker position and decreased in value with distance from this peak. A single QTL was hypothesized in this situation, and the peak marker was used to estimate its effect. If two peaks were present on the same linkage group and were more than 50 cM apart, two QTLs were hypothesized, each represented by the molecular marker at the corresponding peak. Multilocus R^2 values were computed as (sums of squares markers)/(total sums of squares) after all non-significant marker loci had been removed. Significant loci pairs were tested for epistatic interactions using MINITAB's GLM analysis and two-way ANOVA.

Table 1 Designation and description of quantitatively measured traits in apple seedlings

Trait	Progeny	Designation	Min.	Mean (SD)	Max.	Description
Height						Length of the central leader measured from the soil surface to the terminal bud
Height increment (cm)						
Year 1	NY 86229	Y1 HI	8.0	32.9 (10.6)	73.0	Year 1 height
Year 2	NY 86229	Y2 HI	18.9	66.5 (19.7)	110.7	Year 2 height – year 1 height
Year 3	NY 86229	Y3 HI	0.0	46.6 (17.3)	89.0	Year 3 height – year 2 height
Internode number						
Year 1	NY 86229	Y1 IN	10	21(8)	54	The number of internodes on the central leader
Year 2	NY 86229	Y2 IN	8	44 (14)	80	produced from 1 year's growth
Internode length (cm)						
Year 1	NY 86229	Y1 IL	0.7	1.6 (0.5)	3.0	Year 1 height increment/year 1 internode number
Year 2	NY 86229	Y2 IL	0.7	1.5 (0.3)	2.6	Year 2 height increment/year 2 internode number
Base diameter (mm)						
Year 9	NY 86229	BD	21.8	56.9 (16.2)	100.2	Base diameter measured 10 cm above the soil
Base diameter increment (mm)						
Year 1	NY 86229	Y1 BDI	2.6	5.7 (1.3)	9.3	Year 1 base diameter
Year 2	NY 86229	Y2 BDI	0.0	4.3 (2.0)	10.2	Year 2 base diameter – year 1 base diameter
Year 3	NY 86229	Y3 BDI	0.3	6.0 (2.5)	14.2	Year 3 base diameter – year 2 base diameter
Branch number						
Year 3	NY 86229	BN	1	11 (8)	42	Number of branches arising from the central leader
Leaf break						
	NY 86229	LB	14	17.0 (1.6)	22	Described in Materials and methods

Results

Phenotypic data

Tree vigor was assessed using tree-height and base-diameter measurements taken after the first 3 years of growth. A measure of the growth produced in a season was obtained by subtracting the previous year's measurement from the current value. Minimum values of zero were obtained for several traits, indicating that at least one tree produced no measurable growth that season (Table 1).

NY 86229 is an F_1 progeny produced from a cross between domestic apples. Apple cultivars show high levels of heterozygosity, and there was a large degree of phenotypic variation in the progeny for all traits studied, with no discrete groupings. Minimum and maximum values greater than two phenotypic standard deviations were observed for all traits except Y1 IN, Y1 IL, and BN (abbreviations defined in Table 1). All traits had approximately normal distributions except for BN which was strongly skewed towards the larger values (data not shown). A log-transformation improved normality for this trait and the transformed data were used for QTL analysis.

Strong correlations occurred between HI and BDI in years 2 and 3 (Table 2). BDI and HI were nearly equivalent in predicting mature tree size as measured by base diameter in year 9, with correlations increasing for each successive year to about 0.6 in year 3. IL and IN were moderately correlated to Y1 HI. However, in year 2, IN was strongly correlated to HI, but IL was not. IN and IL were negatively correlated.

QTL analysis

Height increment

Five, three, and four genomic regions were associated with height increment in years 1, 2, and 3 respectively (Table 3). Putative QTLs were found on seven different linkage groups. The percentage of phenotypic variation explained by linear regression on individual markers ranged from 3.9 to 7.9%. Associations between Y1 HI and markers were found in similar positions on linkage group 12 in both parental maps, suggesting that both parents were heterozygous for a common QTL. No genomic regions were associated with HI in all 3 years, although four were in 2 of the 3 years (LGs 7, 9, 10, and 12). When all markers were combined into a multi-locus model, 18.9, 15.6, and 18.9% of the phenotypic variation could be explained for Y1, Y2, and Y3 HI, respectively.

Internode length and number

Three putative QTLs were found for Y1 IL and two for Y2 IL (Table 3). Multilocus models explained 37.0 and 12.9% of the phenotypic variation for Y1 IL and Y2 IL, respectively. Only the region on LG 9 was detected in

Table 2 Correlation coefficients (*r*) among vigor-related traits from progeny NY 86229 $r = 0.152$ at $P = 0.05$, $r = 0.195$ at $P = 0.01$; 170 *df*

	Y1 HI[a]	Y2 HI	Y3 HI	Y1 IN	Y2 IN	Y1 IL	Y2 IL	Y1 BDI	Y2 BDI	Y3 BDI	BD
Y1 HI	—										
Y2 HI	− 0.003	—									
Y3 HI	− 0.003	0.374	—								
Y1 IN	0.6	0.3	0.1	—							
Y2 IN	− 0.1	0.8	0.3	0.2	—						
Y1 IL	0.513	− 0.249	− 0.038	− 0.387	− 0.359	—					
Y2 IL	0.256	0.095	0.089	0.202	− 0.435	0.157	—				
Y1 BDI	0.212	0.389	0.196	0.620	0.340	− 0.411	0.066	—			
Y2 BDI	0.048	0.705	0.359	0.172	0.532	− 0.102	0.141	0.080	—		
Y3 BDI	0.071	0.480	0.639	0.231	0.409	− 0.136	0.120	0.312	0.569	—	
BD	0.156	0.376	0.572	0.090	0.136	0.120	0.356	0.156	0.468	0.625	—
LB	0.206	0.182	0.057	0.232	0.085	− 0.013	0.133	0.166	0.0168	0.131	0.238

[a] Trait abbreviations defined in Table 1

both years. Three putative QTLs were detected for Y1 IN and two for Y2 IN (Table 3). Multilocus models explained 29.8 and 11.3% of the phenotypic variation for Y1 IN and Y2 IN, respectively. A single region on LG 10 was associated with internode number in both years.

Base diameter increment

The number of putative QTLs detected for base diameter increment in NY 86229 varied from one in year 2 to five in year 3 (Table 3). Seven different linkage groups had regions associated with base diameter. Individual locus R^2 values ranged from 4.0 to 14.7% of the phenotypic variation explained. The multilocus models explained 17.5, 6.6, and 24.4% of the variation for Y1, Y2, and Y3 BDI, respectively. A single region on LG 10 was detected in more than 1 year.

Base diameter

Base diameter was recorded in 1995 to provide a measure of total tree size after several years of growth. QTL analysis was then performed on the data to find regions associated with mature tree size. Two regions were associated with BD, with R^2 values of 5.5 and 7.5%. The multilocus model explained 11.6% of the phenotypic variation. The genomic region on LG 7 was also associated with Y1 and Y3 HI, Y1 IN, Y3 BDI, BN and LB (Fig. 3). The genomic region on LG 9 was also associated with Y1 HI, Y1 and Y2 IL, and Y2 HI.

Branch number and leaf break

Two genomic regions were associated with branch number, explaining 7.1 and 24% of the phenotypic variation (Table 3). The multilocus model explained 25.5% of the variation. Seven genomic regions on six linkage groups were associated with leaf break (Table 3). Individual R^2 values ranged from 3.9 to 7.3%, and the multilocus model explained 41.6% of the phenotypic variation. These results are consistent with those of Murawski (1967) who found the time of leaf bud break to exhibit a continuous distribution, consistent with control by several genes.

Epistatic interactions

All markers significantly associated with a trait were tested for digenic epistatic interactions with all other markers significantly associated with that trait. At a probability level of $P \leqslant 0.01$ no significant interactions were detected.

Discussion

QTL analysis using RAPD markers and a double pseudo-testcross mapping scheme

QTL mapping studies commonly make use of F_2 or backcross progenies derived from inbred lines. Severe inbreeding depression and self-incompatibility make the development of such progenies impractical in apple. A substitute mapping method employed is the double pseudo-testcross mapping method (Weeden 1994). In this procedure, highly heterozygous cultivars are crossed and independent maps are made for the loci segregating from each parent. Because RAPDs are dominant, two types of segregation are generated using this format. Markers heterozygous in only a single parent segregate in a 1 : 1 present : absent ratio in the progeny, and markers heterozygous in both parents segregate in a 3 : 1 present : absent ratio. Doubly heterozygous markers are less informative than singly heterozygous markers because the dominant-allele progeny are composed of three indistinguishable genotypes:

Table 3 Location, significance level, and estimated effect of putative QTLs in NY 86229

Trait[a]	Linkage group	Map Postition[b]		Marker	*P* value[c]	R^2 (%)[d]	Genotype means (SD)[e]		Δ_1[f]	Δ_2[g]
		NY-58	WM				(+)	(−)		
Y1 HI (cm)										
	6	51 (−)	69 (−)	P122x	0.002	6.1	34.6 (10.3)	27.6 (9.3)	0.66	0.16
	7	–	57 (−)	B318w	0.000	7.9	36.1 (9.6)	30.1 (10.8)	0.57	0.30
	9	59 (+)	31 (−)	B268x	0.001	6.8	34.1 (10.0)	27.3 (9.8)	0.64	0.11
	12	–	35 (−)	B205b	0.007	6.4	30.3 (8.3)	35.5 (11.6)	0.49	0.25
	12	22 (+)	–	B301b	0.01	5.9	35.0 (10.2)	29.9 (10.2)	0.48	0.20
Y2 HI (cm)										
	9	51 (+)	–	B523c	0.003	7.5	76.0 (17.8)	65.9 (16.8)	0.51	0.48
	10	–	27 (−)	P255a	0.005	4.5	69.8 (19.1)	61.4 (19.6)	0.43	0.17
	21	43 (+)	–	B302z	0.008	4.2	71.3 (18.9)	63.2 (19.8)	0.41	0.24
Y3 HI (cm)										
	7	–	57 (−)	B318w	0.01	3.9	50.4 (16.5)	43.6 (17.6)	0.39	0.22
	10	–	26 (−)	B180b	0.002	5.3	50.1 (16.6)	42.1 (17.3)	0.46	0.20
	11	–	21 (−)	B403a	0.001	7.1	42.4 (17.7)	51.5 (15.6)	0.53	0.28
	12	–	41 (+)	B536c	0.005	4.6	43.2 (15.7)	50.5 (17.8)	0.42	0.23
Y1 IL (cm)										
	6	38 (−)	54 (−)	P153z	0.004	6.2	1.66 (0.53)	1.33 (0.44)	0.66	0.12
	9	–	4 (−)	S12y	0.000	8.2	1.78 (0.56)	1.47 (0.48)	0.62	0.36
	10	–	69 (−)	P459z	0.000	23.1	1.39 (0.40)	1.91 (0.57)	1.04	0.62
Y2 IL (cm)										
	5	–	57 (−)	S23z	0.010	4.6	1.62 (0.34)	1.49 (0.23)	0.43	0.40
	9	59 (+)	31 (−)	B268x	0.000	8.0	1.59 (0.30)	1.37 (0.27)	0.73	0.30
Y1 IN										
	1	8 (−)	–	S34c	0.007	4.3	23.1 (8.9)	19.8 (6.4)	0.41	0.26
	7	–	57 (−)	B318w	0.001	6.3	23.6 (8.9)	19.7 (6.3)	0.49	0.33
	10	–	69 (−)	P459z	0.000	16.8	24.1 (8.8)	17.8 (3.9)	0.79	0.39
	12	–	35 (−)	B205b	0.007	6.5	20.7 (6.1)	24.9 (9.7)	0.53	0.49
	21	11 (+)	–	B536x	0.005	4.6	23.4 (9.2)	20.0 (5.9)	0.43	0.30
Y2 IN										
	5	42 (−)	–	OB15z	0.003	5.0	47.1 (14.2)	40.9 (12.9)	0.44	0.22
	10	–	69 (−)	P459z	0.001	6.9	47.0 (13.9)	40.1 (12.8)	0.49	0.21
Y1 BDI (mm)										
	10	–	69 (−)	P459z	0.000	14.7	6.1 (1.2)	5.2 (1.1)	0.69	0.31
	15	15 (−)	36 (−)	B269b	0.002	5.7	5.5 (1.1)	6.2 (1.4)	0.54	0.38
Y2 BDI (mm)										
	21	43 (+)	–	B302z	0.001	6.6	4.9 (2.2)	3.9 (1.8)	0.50	0.23
Y3 BDI (mm)										
	2	27 (+)	44 (−)	B319w	0.004	4.9	5.7 (2.2)	6.8 (2.9)	0.44	0.32
	7	–	57 (−)	B318w	0.002	5.8	6.7 (2.8)	5.4 (2.1)	0.48	0.28
	10	–	69 (−)	P459z	0.001	6.1	6.6 (2.7)	5.3 (2.1)	0.52	0.24
	14	24 (−)	–	OB18y	0.008	4.0	5.5 (2.2)	6.5 (2.7)	0.28	0.20
	16	–	0 (+)	P255b	0.000	8.5	6.8 (2.7)	5.3 (2.1)	0.60	0.32
Y9 BD (mm)										
	7	–	57 (−)	B318w	0.004	5.5	60.8 (15.7)	53.3 (15.6)	0.46	0.24
	9	56 (+)	27 (−)	B319y	0.001	7.5	59.1 (16.3)	47.6 (11.7)	0.71	0.14
BN										
	7	–	35 (+)	B474f	0.000	7.1	12.8 (8.5)	8.5 (6.0)	0.55	0.31
	10	–	69 (−)	P459z	0.000	24.3	7.8 (6.9)	15.0 (7.1)	0.90	0.40
LB										
	3	70 (−)	–	B150c	0.006	3.9	16.6 (1.5)	17.3 (1.7)	0.44	0.19
	6	77 (−)	94 (−)	P126x	0.007	6.5	17.4 (1.7)	16.5 (1.6)	0.56	0.25
	7	–	23 (+)	B474f	0.008	4.7	17.4 (1.7)	16.7 (1.5)	0.44	0.25
	9	11.4 (+)	–	B319z	0.001	5.4	17.6 (1.7)	16.7 (1.6)	0.56	0.38
	9	101 (−)	83 (+)	B423y	0.004	7.3	16.7 (1.7)	17.7 (1.6)	0.63	0.44
	11	–	32 (+)	B474b	0.002	4.9	16.7 (1.6)	17.4 (1.7)	0.44	0.25
	12	38 (+)	–	B303x	0.004	6.0	17.6 (1.7)	16.8 (1.6)	0.50	0.38
	15	–	43 (+)	B523b	0.007	7.3	17.4 (1.8)	16.6 (1.5)	0.50	0.25

[a] For a description of traits see Table 1
[b] Position of the marker in the parental map; numbers represent the distance in cM from the top marker on the linkage group as depicted in Conner et al. (1997). (+) and (−) indicate coupling or repulsion phase of marker respectively in reference to the first marker linkage group
[c] Significance level of marker in multilocus model
[d] % phenotypic variation explained; SS marker/SS total from single-factor ANOVA
[e] Mean and standard deviation of band-present progeny (+) and band-absent progeny (−)
[f] Difference between alternative QTL genotypes expressed in phenotypic standard deviations
[g] Difference between the favorable QTL genotype (more vigorous growth, earlier leaf break, and fewer branches) and the population mean expressed in phenotypic standard deviations

$++$, $+-$, and $-+$. However, doubly heterozygous markers are valuable in this mapping scheme because they allow homologous linkage groups from the parental maps to be aligned through the use of "locus bridges" (Echt et al. 1993). In this mapping population about one-third of the markers were doubly heterozygous (Conner et al. 1997).

The use of the double pseudo-testcross mapping scheme in combination with dominant RAPDs presents challenges in both the ability to detect QTLs and in describing their genetic effects. QTL mapping studies that use a pseudo-testcross format differ from those that use inbred populations in that up to four different QTL alleles may be present in the two parents. Because the two parents were not derived from the same F_1 individuals, the QTL alleles in each may differ. If the genotype of one parent is Q_1Q_2 at the QTL locus, and the other parent is Q_3Q_4, for singly heterozygous markers, the QTL analysis tests the difference between the average trait value of $(Q_1Q_3 + Q_1Q_4)$ versus $(Q_2Q_3 + Q_2Q_4)$ in the first parent, and $(Q_3Q_1 + Q_3Q_2)$ versus $(Q_4Q_1 + Q_4Q_2)$ in the second parent (Grattapaglia et al. 1995). Thus, the quantitative value of alternative marker genotypes is measured as the effect of one allelic substitution averaged over potentially two alternative alleles inherited from the other parent. In the case of a QTL linked to a doubly heterozygous marker, with band-absent marker alleles linked to Q_2 and Q_4, the QTL analysis tests the difference between the trait value of (Q_2Q_4) versus the average of $(Q_1Q_3 + Q_1Q_4 + Q_2Q_3)$. Specific intralocus interactions can not be tested, because the QTL genotype of each parent is unknown and the information provided by dominant markers is limited. As a result, more variation exists in this system because the effect of the QTL allele substitution is measured against a heterogeneous background. Therefore, this study may have been limited to detecting loci with specific genetic configurations and/or strong effects. In addition, the additivity/dominance ratio of QTLs could not be estimated, either due to the lack of a homozygous dominant class or because the homozygous dominant class could not be differentiated from the heterozygote.

Despite the limitations inherent in our mapping scheme, associations were found between markers and each of the traits analyzed. The number of putative QTLs detected for each trait varied from eight for LB to only one for Y2 BDI, with an average of three QTLs detected for each trait. Individual R^2 values ranged from 3.9 to 24.3%, with an average of 7.2% of the phenotypic variation explained. Multilocus R^2 estimates for traits ranged from 6.6 to 41.6% with an average of 24.3% of the variation explained by all the putative QTLs detected for a trait. These R^2 values may be reduced because the effect of QTL allele substitution was measured against a heterogeneous background, thus increasing the marker-class errors and reducing R^2 values.

The effect of QTL alleles on trait performance was also estimated by the difference between genotype classes (Δ_1) and the difference between the favorable marker class (Δ_2) and the trait mean measured in trait phenotypic standard deviations. Grattapaglia et al. (1995) suggested that the percent variation explained may not be the most useful measure of the importance of a QTL from a marker-assisted selection standpoint. Measurement of Δ_2 may provide a more useful estimate of the potential gain to be realized from the selection of a favorable QTL marker allele. Values of Δ_2 ranged from 0.11 to 0.62 (Table 3). Alleles with the highest R^2 values are not necessarily the alleles with the largest Δ_2 values. This effect is most pronounced when the favorable allele of a QTL is linked to the band-positive phenotype of a doubly heterozygous marker. In this case, progeny with the marker present will consist of a heterogeneous mixture of three genotypes ($++$, $+-$, and $-+$). For example, marker B319y, where the favorable allele is linked to the band-present phenotype, explains a greater proportion of the phenotypic variation for BD than marker B318w, 7.5% vs 5.5% (Table 3), but has a much lower Δ_2 value, 0.14 vs 0.24, and thus would be less useful in selecting for increased BD. Doubly heterozygous markers will be most effective in selection if the trait of interest is linked to the band-absent phenotype, since all progeny in this class will have the favorable QTL allele. However, the potential gain from selection of the homozygous-absent phenotype will be partially offset by a decrease in selection efficiency as only one-quarter of the progeny will be homozygous-absent while one half of the progeny inherited the favorable allele from the respective parent.

Genetic analysis of traits

One of the most useful aspects of QTL studies in woody plants may be the ability to dissect the genetic components of complex quantitative traits (Strauss et al. 1992; O'Malley 1996). Correlated traits often have QTLs that map to similar genomic locations (Paterson et al. 1991; Xiao et al. 1995). This apparent clustering of QTL alleles can be due either to tight linkage between QTLs

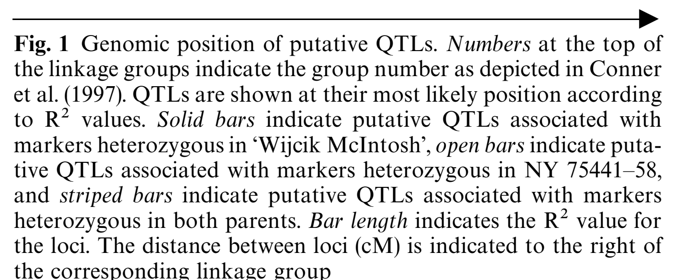
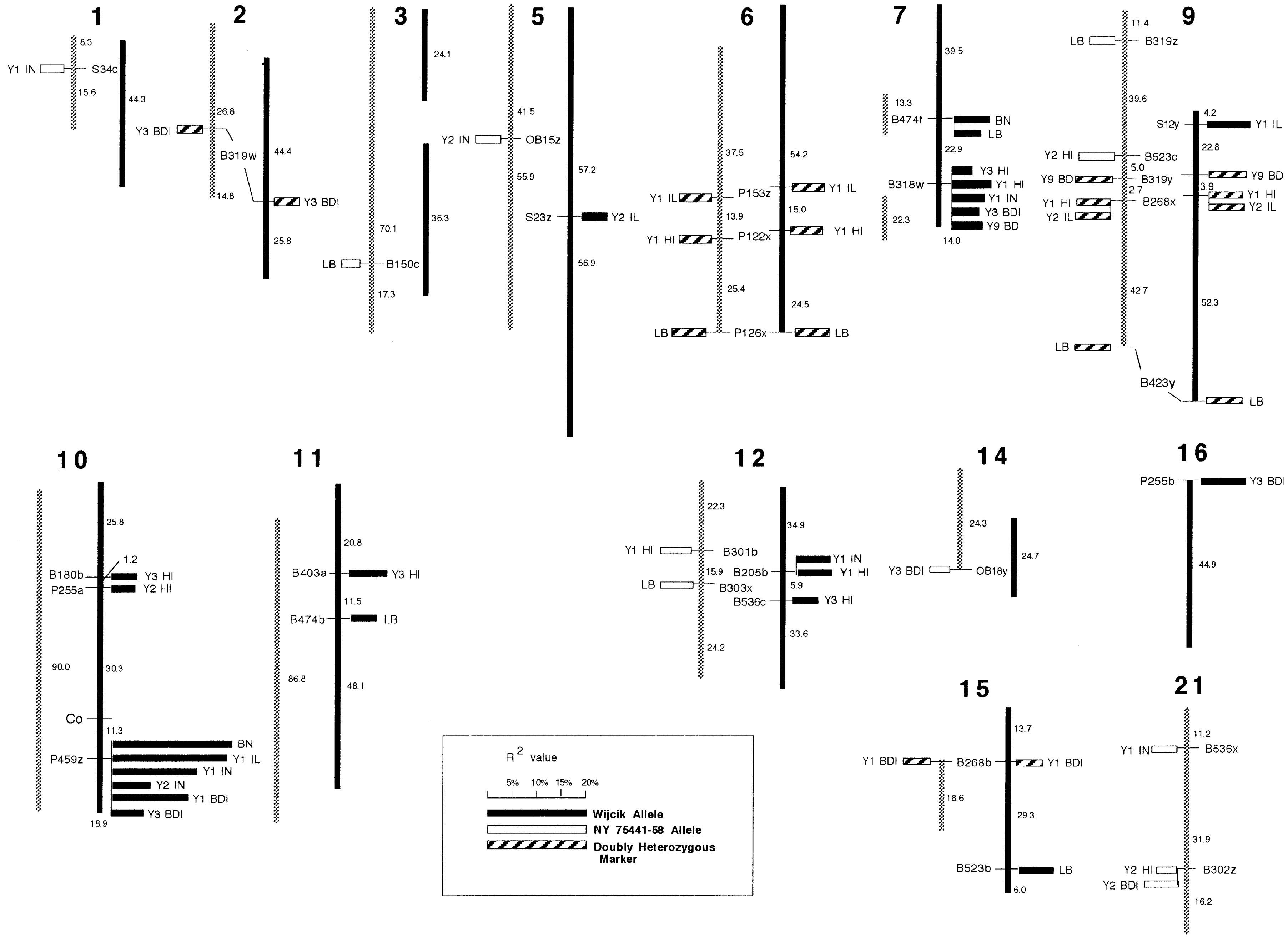


Fig. 1 Genomic position of putative QTLs. *Numbers* at the top of the linkage groups indicate the group number as depicted in Conner et al. (1997). QTLs are shown at their most likely position according to R^2 values. *Solid bars* indicate putative QTLs associated with markers heterozygous in 'Wijcik McIntosh', *open bars* indicate putative QTLs associated with markers heterozygous in NY 75441–58, and *striped bars* indicate putative QTLs associated with markers heterozygous in both parents. *Bar length* indicates the R^2 value for the loci. The distance between loci (cM) is indicated to the right of the corresponding linkage group



1
Y1 IN
S34c
2
Y3 BDI
B319w
Y3 BDI
3
LB
B150c
5
Y2 IN
OB15z
S23z
Y2 IL
6
Y1 IL
P153z
Y1 IL
Y1 HI
P122x
Y1 HI
LB
P126x
LB
7
B474f
BN
LB
B318w
Y3 HI
Y1 HI
Y1 IN
Y3 BDI
Y9 BD
9
LB
B319z
S12y
Y1 IL
Y2 HI
B523c
Y9 BD
B319y
Y9 BD
Y1 HI
B268x
Y1 HI
Y2 IL
Y2 IL
LB
B423y
LB
10
B180b
Y3 HI
P255a
Y2 HI
Co
P459z
BN
Y1 IL
Y1 IN
Y2 IN
Y1 BDI
Y3 BDI
11
B403a
Y3 HI
B474b
LB
12
Y1 HI
B301b
B205b
Y1 IN
Y1 HI
LB
B303x
B536c
Y3 HI
14
Y3 BDI
OB18y
15
Y1 BDI
B268b
Y1 BDI
B523b
LB
16
P255b
Y3 BDI
21
Y1 IN
B536x
Y2 HI
B302z
Y2 BDI
R2 value
5% 10% 15% 20%
Wijcik Allele
NY 75441-58 Allele
Doubly Heterozygous Marker

or to a single QTL with pleiotropic effects. Differentiating between pleiotropy or QTL clustering is difficult because crossovers between tightly linked genes will be rare, and population sizes are often insufficient for their detection. However, when the mode of action of the concurrent QTLs is similar, pleiotropy seems to be the most likely explanation. Thus, QTL studies may help us to better understand the relationship between the plant growth and architecture traits. Lawson et al. (1995) found a QTL for branching habit in apple that was associated with initial vegetative growth, suggesting pleiotropic effects of a single locus. In *Populus*, Bradshaw and Stettler (1995) found QTLs for diameter growth to be closely linked to QTLs for branch traits, providing evidence of single QTLs underlying these traits.

Lawson et al. (1995) reported branching habit in the cross 'White Angel' × 'Rome Beauty' to be highly correlated with segregation at a single locus designated *terminal bearing* (*Tb*). The *terminal bearing* ('Rome Beauty') type featured few or no lateral branches on the lower half of the shoot (blind wood), with most of the fruit buds being born near the ends of the branches. *Tb* maps to LG 6 of the 'White Angel' × 'Rome Beauty' map of Hemmat et al. (1994), which is homologous to LG 10 of our map, and *Tb* maps close ($\pm$ 20 cM) to the position of *Co* (N. Weeden, unpublished data). Lawson et al. (1995) also reported that vegetative bud break was correlated with segregation of the terminal bearing habit, and may be a pleiotropic effect of *Tb*. However, no associations were found between markers on LG 10 and leaf break.

Many of the traits measured in the progeny were strongly correlated with each other (Table 2) and many putative QTLs were clustered on the linkage map (Fig. 1). Strong correlations were found between height and the base-diameter increment in years 2 and 3. On LG 7 and 21 a single marker was associated with both HI and BDI. It seems likely that these are the result of single QTLs with pleiotropic effects on both HI and BDI rather than separate linked genes. However, other regions are only associated with HI or BDI. From this evidence it appears that some QTLs may act in a general fashion, increasing overall tree vigor, with a resultant increase in HI and BDI, while others may be more specific, with significant effects only on HI or BDI.

Large clusters of marker-trait associations occurred on LGs 7, 9, 10, and 12, and smaller groups appeared on LGs 6 and 21 (Fig. 1). The large cluster on LG 10 is very near the mapped position of the columnar gene *Co* that is known to have a large impact on tree growth and form (Lapins 1969). Therefore, it was not surprising to find many traits associated with markers linked closely to *Co* (Conner et al. 1997). This leads to the intriguing possibility that the other large clusters of marker-trait associations may be the result of single loci with pleiotropic effects on many traits.

QTL stability

The most valuable QTLs from a breeder's standpoint will be those that show a consistent positive expression in multiple environments, and over multiple seasons. QTL stability over years was evaluated by comparing the locations of putative QTLs for traits measured in different years. From ten genomic regions associated with HI, none were detected in all 3 years, four were detected in 2 years, and six were detected in only a single year. One out of five regions were asssociated with IL in Y1 and Y2, and one out of seven regions were associated with IN in both Y1 and Y2. Out of eight regions associated with BDI, no associations were detected in all 3 years, and only a single region was associated with BDI in 2 years.

The majority of the putative QTLs for apple vigor were detected in only a single year. Variation in QTLs detected in different years can be the result of QTL × environment interactions, developmentally regulated QTLs, or chance fluctuations in the data sets in different years. QTLs with environmental specificity and QTLs active over diverse environments have been reported in annual crops (Paterson et al. 1991; Stuber 1992; Bubeck et al. 1993; Schön et al. 1993; Lee et al. 1996). In year 1, seedlings were grown for several months in the greenhouse and then transplanted into the field, resulting in environmental conditions very different from years 2 and 3. Developmentally regulated QTLs for plant height have been reported in maritime pine seedlings grown for an extended period in the greenhouse (Plomion et al. 1996). Two kinds of QTLs were observed, those showing a continual expression during plant growth, and those detected only at one stage of plant growth, suggesting that maturation induces a progressive shift of the genetic control of height growth. Developmentally regulated QTLs have also been shown for plant height in maize (Edwards et al. 1992) and in *Brassica rapa* (Song et al. 1995).

Much of the between-year variation observed in this study may be a result of the low statistical power of the QTL detection methods employed. Further testing of QTLs in replicated progenies would help to clarify the cause of the variation between years, and the ability to clonally propagate apple seedlings provides prospects for such studies. Two genomic regions were associated with Y9 BD (LG7 and 9; Table 3), indicating that some QTLs may be expressed consistently enough to influence mature tree size. Both of these regions were associated with HI in multiple years (2 out of 3). However, other regions also associated with HI in 2 years (LG 10 and 12) did not have an effect on BD. Measuring mature trees might then be the best method of detecting those QTLs which will be most useful in selecting tree vigor. However, QTL analysis of individual growing seasons may provide valuable insights into the genetic regulation of tree growth.

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