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Resolution of quantitative trait loci for mechanical measures accounting for genetic variation in fruit texture of apple (*Malus pumila* Mill.)

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Abstract Different attributes of fruit flesh texture contribute to consumer preference for eating quality in apple. We extended previous studies in order to determine whether a range of mechanical measures could resolve further the genetic contribution to variation in the physical attributes of apple fruit flesh. In particular we were interested in accounting for variation of the sensory traits crispness and juiciness. A quantitative genetic analysis of mechanical measures derived from compression and wedge fracture tests was carried out. This was based on segregation in an unselected mapping population which had previously been used to identify QTLs associated with penetrometer readings, stiffness by acoustic resonance, and a range of sensory descriptors. For wedge fracture tests significant QTLs were detected on L16 and L01. Those on L16 corresponded with positions previously determined for sensory measures of crispness and juiciness. Distance at maximum force was accounted for by a single QTL on L16 and correlated well with crispness and juiciness, suggesting that it may be appropriate for the selection of genotypes with fruit posessing desirable texture attributes. We established that the association of the sensory texture QTL on L16 is unlikely to be due to perceptual interactions with the *Ma* acidity locus. For compression measures, QTLs were detected on L01, L06, L08 and L15. Specific gravity is well-correlated with compression stiffness modulus, and both have a significant QTL on L06. Measures of cell size and shape determined across the population failed to detect any significant QTLs.

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Introduction

The fruit flesh of the dessert apple, *Malus pumila* (Mill.), consists of a cortex of cells radially elongated with variation in the degree of radial airspaces (Khan and Vincent 1990). Cell size increases from close to the region of the vascular bundles towards the outer cortex, where cell size then reduces and cells become more spherical (Bain and Robertson 1951). Variation in the texture of this tissue is a major component of consumer preference for eating quality. Characterising the genetic contribution to variation in fruit texture will provide both information and molecular markers to increase the efficiency of selective breeding programmes, as well as indicating the possible contribution of candidate genes isolated from other studies. Recently, we identified QTLs on seven of the 17 *Malus* linkage groups, which accounted for variation in firmness as determined by the Magness-Taylor penetrometer (Magness and Taylor 1925), stiffness determined by acoustic resonance, and sensory attributes of texture assessed by a trained panel (King et al. 2000). The analysis was based on an unselected segregating population, replicated at several sites, for which a detailed linkage map had previously been constructed (Maliepaard et al. 1998).

Whilst the mechanical measures used in that work are more amenable to the large-scale objective screening of fruit required for breeding and genetic studies, it is important to establish the relationship between the physical measurements and sensory, and thus consumer, perception of texture. Fruit firmness, as determined by penetrometer, measures the force required to press a blunt-ended probe into the tissue. The resistance to penetration is a combination of compression stiffness and strength and shear strength, and represents a compound of largely uncontrolled deformations and failures. In earlier work we found that the penetrometer measurement had a cor-

relation of 0.63 with the sensory descriptors of hardness and slow breakdown, and of 0.53 with crispness, whereas the stiffness assessed by acoustic resonance was poorly correlated with these and other measures. The complexity of the penetrometer measure was also reflected in QTL analyses, where LOD scores greater than 3.0 in at least one environment were observed for 12 of the 17 linkage groups. Significant QTLs were observed for five linkage groups.

Sensory assessment has also been successful in detecting QTLs representing different attributes of fruit texture. For one or more of the sensory descriptors LOD scores greater or equal to 3.0 were found for nine linkage groups. A highly significant QTL was detected on L16 for the descriptors crispness, juiciness, sponginess and overall liking. Although this QTL was not detected by either penetrometer or acoustic resonance measures, it did map in coupling to the region of *Ma*, the malic acid gene (Maliepaard et al. 1998). This close linkage could be explained by one or more of three possible scenarios. The association may be due to perceptual interactions between acidity and texture attributes, it may indicate that there are pleiotropic effects of a gene at the *Ma* locus, or there may exist a cluster of different genes colocalised in the same region. It was hoped that analysis with more-detailed objective measures might provide additional information about this locus.

Mechanical properties of fruit tissue may be assessed by a range of measures routinely used in materials testing. Using uniaxial compression and crack opening tests, Khan and Vincent (1993a, b) found large differences in compression stiffness, failure stress, fracture toughness and failure force between apple varieties which had been selected for their contrasting textural attributes. Measurements made using these types of tests have been shown to be related to the sensory perception of texture. In a study of mealiness in apple, Barreiro et al. (1998) found that crispness and juiciness could be assessed from confined compression of fruit cylinders and acoustic impulse response. In addition they found high correlations between sensory descriptors and instrumental parameters. Compression stiffness and strength were correlated with crispness in Golden Delicious and Granny Smith apples (Lurie and Nussinovitch 1996). Sensory analysis has profiled mealiness as a loss of crispness, hardness and juiciness, with an increased floury sensation in the mouth (Barreiro et al. 1998).

The purpose of the present study was to determine whether we could use a range of mechanical measures to resolve further the genetic contribution to variation in the physical attributes of apple fruit flesh. Our primary aim was to identify significant QTLs which might account for distinct traits, and particularly those associated with the desirable sensory trait of crispness. In addition, we were interested in determining whether there may be a direct relationship between genetic variation for mechanical and sensory measures and the cellular structure of the fruit cortex, since it is known that the morphological components in apple parenchyma greatly influence its mechanical properties (Vincent 1989). We therefore carried out a quantitative genetic analysis of cell parameters on the same population.

Materials and methods

Plant material

The segregating population, which derived from a cross between ‘Prima’ and ‘Fiesta’, has been described elsewhere (King et al. 1998, 2000; Maliepaard et al. 1998). The trees used in this study were grown on M27 rootstock either staked in rows 2-m apart (Elst at, 51′ 55″N 05′ 50″E, 8-m altitude) or in cordons 1-m apart (East Malling at 51′ 17″N 00′ 27″E, 32-m altitude). The positions of trees were randomised between sites to reduce neighbour effects.

Harvesting and sampling

For the mechanical assessments, fruit from the segregating population and parent lines were obtained from Elst in 1997, and from East Malling in 1998. One hundred and thirty individual segregating genotypes were sampled in total, of which a subset of 67 plus parent lines were sampled from both sites. At Elst there were duplicate trees of one parent line (‘Prima’). Fruit were harvested up to twice a week as they reached a ripe harvestable stage. This is an operational decision based on the ability of the fruit to separate readily from the tree at the abscission zone between the stalk and the branch, and is designed to minimise variation known to be present due to differential ripening rates within and between trees. Fruit which were damaged from hail, insects or birds, or which were asymmetrical, were rejected. In general, fruit of a median size were picked, with selection against particularly small or large fruits. Fruit were over-sampled in the field, to allow for possible damage or decay in transit, and were placed in standard apple trays, with smaller fruit individually wrapped in tissue paper. From the field, boxes of apples were stored at 4°C for up to 3 days, and transported by air or land courier as required for assessment at East Malling. Since variance can not be attributed to either site or year, for convenience this is referred to throughout the paper as the environmental effect. For the cellular assessments, fruit were obtained from East Malling in 1997.

Trait assessments

Six fruit per tree were sampled. The weight of whole un-peeled fruit analysed by mechanical measures was recorded, and the specific gravity of the tissue was calculated from the weight of the tissue plug used for compression.

Mechanical properties of fruit flesh tissue

Fruit were cut from stalk to calyx into two sections. Two types of sample were excised from tissue 3–5 mm below the skin of each section. Sample type A was a radially orientated 10-mm cube cut from the equatorial region with a double-bladed knife. Sample type B was a radially orientated 15-mm-diameter 10-mm-long plug, cut from the equatorial region, with a cork-borer which had been sharpened on the outside.

Wedge fracture tests were carried out using a Lloyd Instruments model LRX fitted with a 50N load cell and running with Rcontrol software v3.23. A wedge (with a 30° included angle) was driven at 33.3×10^{-3} mm s^{-1} into tissue of sample type A. Wedge movement was halted when a crack was visible ahead of the wedge tip (after peak force had been reached), and the total crack length was measured. The wedge was then withdrawn from the sample at the same speed so that the energy still stored in the sam-

ple could be subtracted from the total energy. Maximum force, force and distance (of the wedge tip from the top of sample) at the start of the crack propagation were determined from the force-distance curves. In addition, work of fracture was calculated as:

(area under the force-distance curve)/(total crack length × sample width).

Compression tests were carried out using an Instron 1140 fitted with a 500N load cell. Tissue samples of type B were compressed at 33.3×10^{-3} mm s^{-1}. Compression stiffness modulus was calculated from the slope of the linear region of the force-deflection curve. Peak stress, strain at peak stress, stress and strain at first failure (bioyield) were also determined.

Data for penetrometer and sensory measures for the same individuals in the population were derived from the data sets used by King et al. (2000).

Image analysis of cells in the fruit cortex

Fruit were sampled from East Malling in 1997. Transverse sections of tissue were cut from the subcutaneous region of the fruit, which corresponded with that used in the mechanical measurements. For 16 genotypes the fruit were too soft, and thus could not be sampled for these experiments. A surface replica was prepared by applying nail varnish over an area of at least 1 cm^3. The replicas were stored at room temperature in Petri dishes for up to 4 weeks until analysed. Cell orientation was assessed under a light microscrope with a ×10 objective. Two fruit per genotype were sampled, with 25 cells sampled per fruit. The images were captured using a Panasonic three-chip, 24-bit colour camera, and analysed using Optimas software. Values corresponding to area, perimeter, length, breadth, circularity and rectangularity were recorded in µM.

Statistical analysis

All analyses were performed with the statistical package Genstat 5 (Payne et al. 1993). The data from the physical measurements in the 1997 and 1998 experiments were analysed using REML (Patterson and Thompson 1971). REML is an analysis of variance also suitable for unbalanced designs. Data were analysed first with all factors treated as random in order to obtain the variance components. The factors included were crossed effects of genotype and environment, with tree-to-tree differences nested within the genotype x environment interaction, and apple-to-apple differences nested within that. The tree-to-tree term was estimated with one degree of freedom, and for several of the measures the analysis failed to converge with this term included. For these measures, the analysis was performed with this term omitted. Estimates of the proportions of the variance contributed by the various factors were calculated as the ratio of the variance component for that factor to the sum of all variance components. The proportion of variance contributed by the genotype is twice the value of that conventionally referred to as broad-sense heritability. In this calculation, any negative variance components were taken to be zero. In order to obtain estimates of genotype-means for QTL analysis, the analysis was repeated with the genotype treated as fixed.

The 1997 image data were subjected to an analysis of variance. Genotype was taken as a fixed factor, with apple within genotype and cell within apple as random factors.

Principal-components analysis has been used previously to summarise the variation present in complex traits in just a few dimensions prior to QTL analysis (for example, Romagosa et al. 1996; Andersson-Eklund et al. 2000). A principal-component analysis was performed on the genotype estimates from the physical measurements, excluding those for weight and specific gravity. The analysis was performed using correlations rather than variances because the scales of measurement varied. The components accounting for most of the variance were subjected to QTL analysis.

QTL analysis

QTL analysis of the REML and ANOVA estimates for genotype was performed using the Maximum Likelihood-based interval mapping approach of MapQTL ver. 3.0 software (Van Ooijen and Maliepaard 1996), as described in King et al. (2000). The integrated linkage map of ‘Prima’ and ‘Fiesta’ was used (Maliepaard et al. 1998), and direct comparison made with the datasets generated in King et al. (2000). A 1-cM step size was employed.

For interval mapping a LOD score threshold of 3.0 was used to indicate evidence for a QTL. This threshold corresponds to a per-linkage group error rate of 5% for the average linkage group length, which was 63 cM. A threshold of 4.5 was used to indicate significant linkage, which corresponds to a genome-wide error rate of 5% (Van Ooijen 1999). Interval mapping results were checked against results from QTL analysis using the regression approach of Haley et al. (1994), allowing for four alleles of a QTL, and from the non-parametric Kruskal-Wallis test, performed per marker (e.g. Lehmann 1975). Multiple QTL Model (MQM) analysis (Jansen 1994) was performed for a number of traits, using this feature in MapQTL (Van Ooijen and Maliepaard 1996). Marker loci in the vicinity of the *Ma* locus (L16) and the *Vf* locus (L01) were used as cofactors.

Results

Trait distributions

The distribution of trait values across the population displayed evidence of transgressive segregation for all measures (Fig. 1). There were differences in the amount and direction of skewness in the distributions. For compression stiffness modulus, specific gravity and weight (data not presented), ‘Prima’ had lower values than Fiesta’. For all other measures ‘Prima’ had higher values than ‘Fiesta’.

Variance components

There are differences amongst the measures in the amount of variability accounted for by the environment effect (Table 1). This ranges from 0 to 32% dependent upon the measures, and is generally higher for the compression measures than the wedge fracture measures. Between 18 and 46% of the variability is accounted for by the genotype × environment interaction (2 years with a different site each year), with 0 to 39% accounted for by the genotype alone. The compression measure with highest genotype effect is compression stiffness modulus (39%), and the highest genotype effect for wedge measures is the maximum force (30%).

For cellular-structure measures sampled in 1 year, 4–6% of the variation is attributable to genotype, with sample to sample variation being smaller. Most of the variation is attributable to cell-to-cell within a sample, probably as a result of sampling different cellular cross sections within one plane.

Relationships between measurements

A scatter-plot matrix of a selection of the different measures across the segregating population is shown in

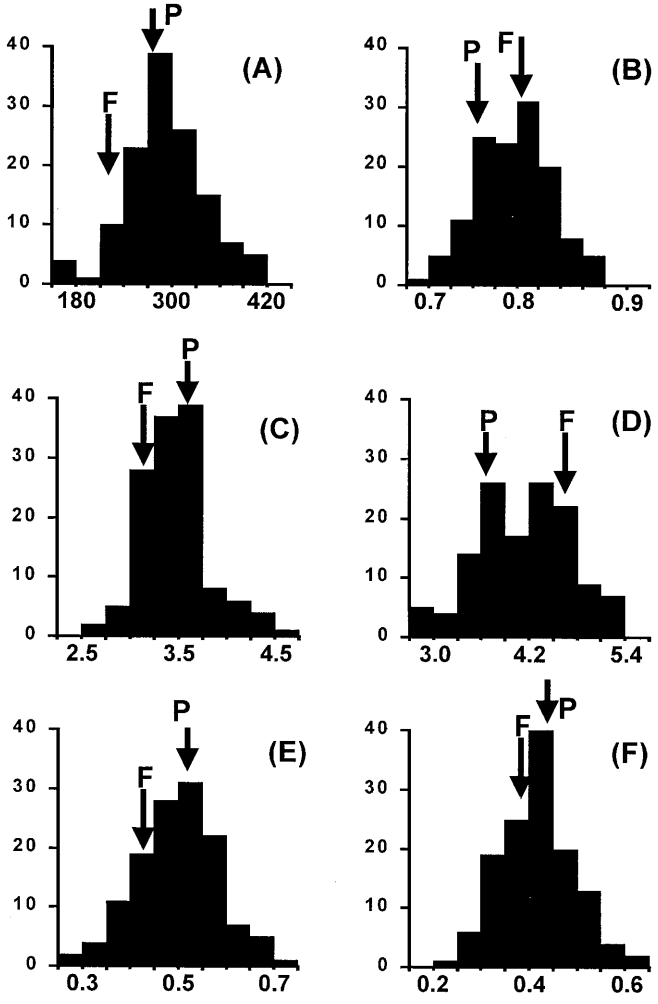

Fig. 1A–F Histograms of genotype estimates for traits relating to fruit texture scored on individuals from the segregating population derived from 'Prima' × 'Fiesta'. Values were calculated using REML. Y axis = number of genotypes in class. Mean parental values are indicated with *arrows*: '*P*' = 'Prima'; '*F*' = 'Fiesta'. **A** Work of fracture ($J.m^{-2}$); **B** specific gravity ($g.cm^{-1}$); **C** distance at crack propagation (mm); **D** compression stiffness modulus (MPa); **E** peak stress (MPa); **F** stress at first failure (MPa)

Fig. 2. Of the wedge measures, maximum force and the force at crack propagation are extremely highly correlated (0.97). Maximum force is highly correlated (0.87) with work of fracture, and correlated with the sensory measure crispness (0.57) and to a lesser extent with juiciness (0.34). The distance at maximum force is most highly correlated with juiciness (0.40). The wedge measure of force at crack propagation is correlated with compression measures (0.5–0.62), specific gravity (0.56), crispness (0.57) and penetrometer reading (0.55).

Compression measures are highly correlated amongst themselves and with specific gravity (0.63–0.77). Stress at first failure and peak stress are very highly correlated. Peak stress and compression stiffness are highly correlated (0.61 and 0.58 respectively) with penetrometer reading, probably reflecting the mode of action of the penetrometer. Compression stiffness modulus is highly correlated with specific gravity (0.77) and crispness (0.5). Cellular measures were poorly correlated with others. However, since 16 genotypes had fruit which were too soft to be assessed for these measures, this sample set was skewed.

The first principal component accounts for 43% of the total variability. It appears to represent a measure of the resistance to fracture of the tissue, with contributions from all measures. The highest weights contributing to this principal component are work of fracture, maximum force, force at crack propagation and peak stress. The second principal component accounts for a further 24% of the variability, and consists primarily of a contrast between compression stiffness modulus, peak stress, and stress at first failure against distance at maximum force and distance at crack propagation. This component thus appears to represent stiffness and compressive strength. The third principal component accounts for 15% of the variability and consists of a contrast between compression stiffness modulus and maximum force, against force, distance and strain at first failure, and strain at peak stress.

QTL analysis

The QTL mapping results are presented in Table 2. For the compression measures, LOD scores greater than 3.0 are observed for five of the 17 linkage groups. Significant linkage (LOD score greater than 4.5) was detected for linkage groups L01, L06, L08, L12 and L15. A possible QTL was detected for stress at first failure on group L13. No QTLs were detected for strain at first failure. For the wedge measures significant linkage is observed on linkage groups L01, L07 and L16. A single QTL on L16 was observed for distance at maximum force and on L01 for force at first failure. For force at crack propagation, and maximum force, QTLs were detected on both linkage groups. Seven additional groups displayed evidence of suggestive linkage, with LOD scores greater than 3.0.

For specific gravity, significant linkage is observed on linkage groups L06 and L16, with possible QTLs detected on a further five linkage groups. For fruit weight, significant linkage is observed on L04, with a possible QTL on L06. Of the cellular parameters, only one possible QTL was detected for circularity, on linkage group L03 (LOD = 3.3). The Kruskal-Wallis test detected some interaction for breadth, length and rectangularity at a significance level of ($p < 0.005$).

Analysis of the first three principal components, which accounted for a total of 82% of the variability, detected significant QTLs on three linkage groups. For the first principal component there were significant QTLs on L01 and L16, and suggestive QTLs on L08 and L15. Significant QTLs were also detected on L16 for the third principal component, as well as on L06. No significant QTLs were detected for the second principal component.

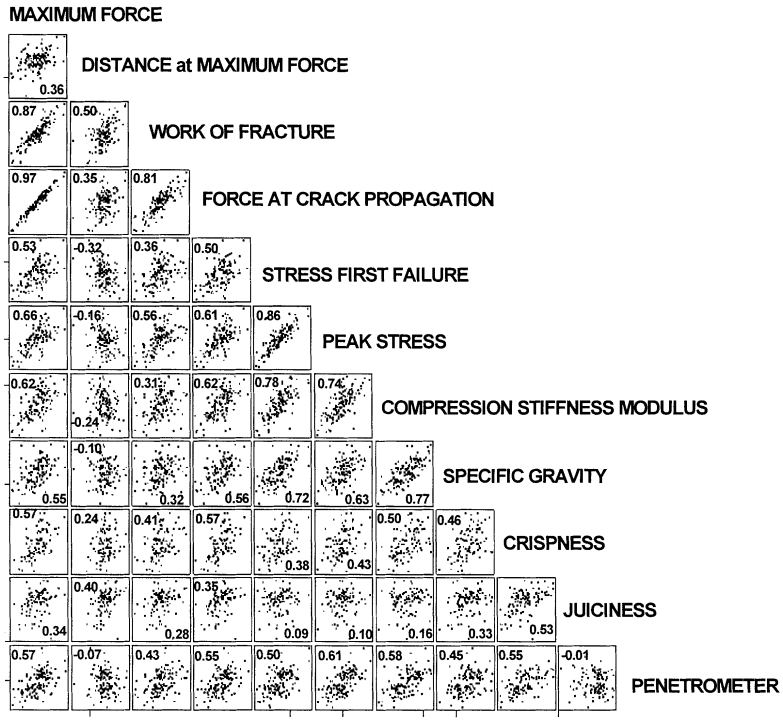

Fig. 2 Scatter plot matrix of mechanical and sensory descriptors. Correlation values have been added to the plots. Individual points correspond to genotype estimates using REML

Table 1 Analysis of components which account for the variance in wedge fracture (W) and compression (C) measures, together with specific gravity, weight and cellular measures. *, ** and *** are significant at 5, 1 and 0.1% respectively. Where a percentage is given as 0 the variance component was negative. Negative variance components were treated as zero in both denominator and numerator when calculating variance components. The term environment is in this case taken as the variance attributed to observations over 2 years, at a different site in each year. The genotype term is equivalent to twice that conventionally referred to as broad-sense heritability. Mean and standard errors for measures are also shown. The residual term corresponds to the apple-to-apple variance. The tree-to-tree variance component is not shown due to poor estimates resulting from the low within-site replication

Measure		Genotype	Environment	Fruit to fruit	Genotype × environment	Residual	Mean	SE
W	Distance at maximum force	19 **	2 **	–	19 ***	59	3.0	0.28
W	Distance at crack propagation	15 **	1 *	–	21 ***	63	3.5	0.34
W	Force at crack propagation	29 ***	7 ***	–	22 ***	42	5.7	0.87
W	Maximum force	30 ***	10 ***	–	28 ***	33	6.1	0.92
W	Work of fracture	19 **	2 **	–	38 ***	41	293	49.6
W	Force at first failure	5 NS	1 *	–	46 ***	47	2.8	0.56
W	Distance at first failure	0 NS	1 NS	–	30 ***	69	0.68	0.08
C	Stress at first failure	24 ***	32 ***	–	18 ***	26	0.42	0.08
C	Peak stress	27 ***	19 ***	–	26 ***	27	0.50	0.09
C	Compression stiffness modulus	39 ***	7 ***	–	18 ***	36	4.2	0.58
C	Strain at peak stress	13 ***	0 NS	–	1 NS	56	0.25	0.05
C	Strain at first failure	19 **	23 **	–	24 ***	33	0.12	0.017
	Specific gravity	64 ***	3 ***	–	10 ***	22	0.80	0.04
	Weight	13 ***	62 ***	–	14 ***	11	118	26.2
	Area	6 ***	–	5 ***	–	89	12,595	2,302
	Breadth	4 ***	–	4 ***	–	92	115.1	10.17
	Circularity	6 ***	–	2 ***	–	92	14.94	0.45
	Length	6 ***	–	5 ***	–	88	149.4	14.6
	Perimeter	6 ***	–	5 ***	–	89	414.6	39.0
	Rectangularity	6 ***	–	1 **	–	93	0.68	0.017

A marker closely linked to *Ma* on L16 was used as a cofactor in MQM analysis, so that this marker would absorb variation due to the significant QTL in this region. For distance at crack propagation, this detected an additional significant QTL (LOD 4.75, accounting for 15%) on L07, as well as a suggestive QTL on L07 for work of fracture. This MQM analysis also increased the LOD score for force at crack propagation and maximum force, for suggestive QTLs on groups L08, L10 and L15. A marker closely linked to *Vf* on L01 was used as a cofactor to absorb variation due to the significant QTL detected for that region. For compression stiffness modulus,

Table 2 QTLs detected with the interval mapping technique for mechanical measurements of wedge fracture (W) and compression (C), together with specific gravity, weight and cellular measures. Previous QTLs detected by King et al. (2000) on the same population for penetrometer readings (FFF), stiffness, and sensory descriptors are also presented. Linkage groups are shown where maximum LOD scores greater than 3.0 were detected (suggestive linkage). Linkage groups with LODs greater than 4.5 are indicated in boxes, with the percentage of variance attributable to the genotype and explained by the putative QTLs indicated below. Kruskal-Wallis tests were carried out for cell measurements and sensory descriptors, and significance levels are indicated by asterisks: * p <0.005; ** p <0.001; *** p <0.0005; **** p <0.0001. [A] Following MQM mapping, with marker M18 on L01 as a co-factor. [B] Following MQM mapping, with marker OPT-16–1000 on L16 as a co-factor. [C] Following MQM mapping, with both OPT-16–1000 on L16 and M18 on L01 as co-factors. Ninf = number of individuals for which data for the quantitative trait were available

Item		Ninf	L01	L03	L04	L05	L06	L07	L08	L10	L12	L13	L14	L15	L16
W	Distance at maximum force	130													6.79 *23%*
W	Distance at crack propagation	130						4.75 [B] *15%*							6.55 *22%*
W	Force at crack propagation	130	6.46 [B] *19%*						4.27 [B]	3.26 [B]				5.32 [C]	9.03 *29%*
W	Maximum force	130	6.47 *21%*		3.85[B]			3.13 [C]	4.38 [B]	3.54[B]				4.29 [B]	9.83 *32%*
W	Work of fracture	130	6.51 *21%*					4.51 [C]	3.47 [B]						6.81 *25%*
W	Force at first failure	130	5.15 17%								3.69				3.24
W	Distance at first failure	130		3.06			3.37								
C	Stress at first failure	130	5.16 *17%*									3.51			
C	Peak stress	130	8.13 *27%*						4.78 *19%*					5.15 [A] *16%*	
C	Compression stiffness modulus	130	5.34 *18%*				4.95 *16%*				4.09 [A]			8.62 [A] *23%*	
C	Strain at peak stress	130					5.23 *19%*								
	Specific gravity	130					7.99 *28%*				3.95	3.61 [B]	4.34 [B]	3.62	4.54 *15%*
	Weight	130			4.53 *25%*		3.12								3.87
PCP	1st Principal component (43%)	130	8.39 *26%*						3.78					3.08	4.45 *16%*
	2nd Principal component (24%)	130		3.67											3.49
	3rd Principal component (25%)	130					4.23 *15%*				3.29			3.69	8.89 *31%*

Table 2 (continued)

Item		Ninf	L01	L03	L04	L05	L06	L07	L08	L10	L12	L13	L14	L15	L16
Cell	Area	74											*		
	Breadth	74				*							*		
	Circularity	74		3.30											
	Length	74	*												
	Rectangularity	74				*									
FFF	Overall	152	6.5 *19%*	3.6					4.7 *16%*	7.4 *22%*				3.5	
	Stiff-overall, day 0	144	4.4							4.5 *16%*					
Sensory	Crispness	113	3.5							3.2	3.9				6.0 *24%*
			*			**	*		**		*	*			****
	Hardness	113				3.7 ***	3.4 **		***	3.7 *	*			3.2	3.8 ***
	Juiciness	113	3.6												14.8 46%

this resulted in a significant QTL being detected on L12 and increased the significance of the QTL on L15. For peak stress the significance of the QTL on L15 was increased. MQM analysis with cofactors for both *Ma* and *Vf* resulted in detection of an additional significant QTL for work of fracture on L07. This analysis also increased the significance (to LOD 5.32) for a QTL detected on L15 for force at crack propagation.

Discussion

The trait distributions for all measures displayed transgressive segregation, consistent with both softer and firmer fruit. 'Fiesta' tissue was stiffer and denser than that of 'Prima', although 'Fiesta' tissue had a lower work of fracture and failed at lower forces and stresses than tissue from 'Prima'. Torsional stiffness of apple tissue has previously been found to increase with tissue density both within and between nine varieties (Vincent 1989). The results are consistent with 'Fiesta' appearing crisper in sensory assessments and more stiff when assessed by acoustic resonance (King et al. 2000), and suggest that 'Prima' may be tougher but not crisper than 'Fiesta'.

The genotype × environment effects were high for most measures. Given the fact that we had the opportunity to sample in 2 years, and the logistics of harvesting, we maximised the estimate of genotype × environment variance by sampling from a different site on each occasion. It is to be expected that such an approach is likely to provide more information about the genotype effects. For wedge fracture measures the environment variance effects were smaller than for compression measures, suggesting that wedge fracture measures may be more appropriate in breeding selection. As the wedge measure of distance at maximum force detected a single QTL on L16 and correlated well with the sensory measures juiciness and crispness, it may be particularly appropriate for the selection of fruit with specific desirable texture attributes. The high genotype × environment effects highlight the difficulties encountered in previous studies when attempting to rank varieties according to texture traits, and emphasise the benefits from accumulating more robust data for a reference segregating population.

We have considerably extended our understanding of the relationships between different mechanical measures and sensory measures. The mechanical measures derived from compression and wedge fracture tests have been successful in distinguishing between different attributes of apple fruit texture. We have found that there are better correlations within measurement type than between wedge and compression measures. Previously, only limited studies had been possible based on single compression tests of individual specimens, which established some very general correlations (Abbott 1994). Earlier genetic studies, which accounted for variation in Magness-Taylor penetrometer and acoustic resonance readings (King et al. 2000), did not detect significant

QTLs corresponding to highly significant QTLs on L16, which had nevertheless accounted for variation in sensory measures such as crispness and juiciness. In this study we have been able to detect correlations between mechanical and sensory measures, both across the population and in the co-location of QTL effects. The correlation coefficients between mechanical and sensory measures observed in this study are considerably lower than established in previous studies which had compared a small number of varieties (Lurie and Nussinovitch 1996; Barreiro et al. 1998). However, our analysis of data from a large number of related genotypes covered a wider range of values.

The relationship between crispness and juiciness and the distance at maximum force is apparent both in the correlation scatter plots (Fig. 2) and the co-location of QTLs to identical regions of L16. As noted previously (King et al. 2000) the region on L16 is also associated with the major locus for fruit acidity (*Ma*). The evidence presented here addresses some of the issues previously raised in terms of possible explanations for the co-location. In particular, the possibility existed previously that the association of sensory texture QTLs on L16 with the *Ma* locus may be due to perceptual interactions. The data suggest that this is now highly unlikely to be the case, since highly significant QTLs accounting for variation in the specific mechanical measures are present in the same region. Two possible scenarios remain, namely pleiotropic effects of a gene at the *Ma* locus, or clustering of genes in the same region. These alternative hypotheses are currently being tested by a more-detailed analysis of recombinants within this region of the genome.

Another aspect of the possible high correlation between some sensory measures has been addressed. In particular, the relationship between crispness and juiciness may arise from the presence of strong inter-cellular bonds in the middle lamella of adjacent cell walls, which when bitten would be more likely to fracture and release juice. In this context, it is interesting to observe that whilst the the compression measures of peak stress and compression stiffness modulus are poorly correlated with juiciness, they are correlated with crispness. Although these correlations are low it should be recognised that the perception of juiciness only requires the cell wall to fracture rather than a failure of intracellular bonds. Thus the tissue need not be stiff nor have high failure stress to result in juiciness. Conversely, the wedge fracture measure of distance at maximum force is less correlated with crispness than it is with juiciness.

Although there appeared to be significant genetic effects associated with variation in a number of the cellular measures, these were not resolved as highly significant QTLs. The premise for testing this hypothesis arose from the observations of Szczesniak and Ilker (1988) who found that the sensory rating for juiciness increased with cell size for several kinds of fruit. More recently, Harker et al. (1997) had suggested that plant tissues which are characterized by juicy textures tend to have large cells which break open during tensile measurements, and that low juiciness was associated with cells which did not break open during tensile measurement.

Specific gravity is well-correlated with compression stiffness modulus, and both have significant QTLs on L06. This is consistent with the findings of Vincent (1989) that torsional stiffness of apple tissue increased with tissue density both within and between nine varieties. Specific gravity, which has a suggestive QTL on L15, is also well correlated with peak stress, which also has a significant QTL on that linkage group.

The significant QTLs detected on linkage group L01 are linked to the introgressed region carrying the locus *Vf*, originating from the scab-resistant crab apple *Malus floribunda*. The allele contributing to and correlated with firmer fruit is in coupling phase with *Vf*, as discussed in King et al. 2000). Bernacchi et al. (1998) have demonstrated the value of introgressing alleles from wild species into cultivated tomato for a range of fruit traits, including firmness, based on earlier QTL data.

The additional resolution obtained by carrying out detailed mechanical measures on a reference segregating population has greatly increased our understanding of the genetics underlying varietal differences in fruit texture. It is apparent that interactions between sensory measures and fruit acidity are unlikely to be due solely to perceptual interactions. We have been able to demonstrate that complex traits such as those contributing to variation in penetrometer readings may be de-convoluted into a series of components. Whilst we have validated the previous findings of a trained sensory panel which assessed this population, it will be important to substantiate these by testing a wider range of genotypes, preferably in other replicated segregating populations. Such analyses will contribute to resolving the issue of whether a relatively small number of key loci are contributing to the variation in fruit texture which is routinely manipulated in modern apple-breeding programmes.

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