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Quantitative genetic analysis and comparison of physical and sensory descriptors relating to fruit flesh firmness in apple (*Malus pumila* Mill.)

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Abstract Texture is a major component of consumer preference for eating-quality in apple. A quantitative genetic analysis of traits associated with fruit-flesh firmness was carried out. This was based on segregation in an unselected mapping population replicated at six sites and harvested over 2 years. Different methods of assessment were compared, and a principal components analysis carried out. Instrumental measures used were Magness-Taylor penetrometer readings, stiffness by acoustic resonance, and a range of sensory descriptors assessed by a trained panel. There were good correlations between some measures, although stiffness was poorly correlated. Whilst genotype by environment effects were large, significant effects were attributable to the genotype, and these were used to detect QTLs. Significant QTLs were detected on seven linkage groups, with large effects on linkage groups L01, L10 and L16. Whilst there was a poor correlation between acoustic stiffness and other measures, the significant and suggestive QTL detected for stiffness on linkage group L10 did represent a subset of significant QTLs detected for the penetrometer measure. The use of sensory assessment proved valuable in detecting QTLs representing different attributes of fruit texture. The possibility of interaction between significant QTLs for fruit texture and other strongly selected traits such as scab resistance and fruit acidity is addressed.

Key words Apple · Fruit · Firmness · Texture · QTL analysis · Genes

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Introduction

Fruit texture is a major component of consumer preference for eating-quality in apples, with appearance and flavour being the other major components. There is a considerable demand for high quality, firm apples that will maintain or achieve their optimal texture properties following harvest, storage, distribution and retailing. In tests conducted by marketing inspectors at harvest, and in breeding selection programmes, texture has often been equated with quality in apples; firm, crisp apples being the ideal. Existing cultivars vary considerably in their innate textural properties, and in their response to various environmental factors. However, it is apparent from breeding programmes, sensory trials and universal anecdotal observation that there is a considerable cultivar-specific, and therefore a genetic, contribution to fruit texture.

Several approaches have been developed to quantify the variation in apple fruit texture, for breeding selection and as an indication of fruit maturity in the production and marketing chain. The fruit of apples consist of an epidermal layer, covering a relatively uniform cortex of parenchyma cells which surrounds a complex inner core of the pericarp and seed. Assessment of texture tends to be focused on the cortex tissue, although this may be influ-

enced by skin, core or fruit size depending on the technique adopted. The physical methods developed include penetrometer readings and non-invasive acoustic resonance measurements to assess fruit stiffness. Sensory evaluation has been carried out either by trained or untrained panels, or else by individual breeders and growers.

The sensory evaluation of apple fruit has played a significant role in pre-release trials as well as in the comparison of modern and existing cultivars. The perception of fruit-flesh texture has been studied in various sensory trials as comprising components of firmness, chewing response and juiciness (Watada and Abbott 1985; Daillant-Spinnler et al. 1996). Due to the large contribution of environmental factors in fruit texture variation, such studies are often difficult to interpret where fruit originate from different orchards or regions, where they differ in harvest time, or when different or untrained sensory panels are employed. Prior to the work reported here, a preliminary study with a trained and stable sensory panel was carried out in 1994 and 1995 (Daillant-Spinnler et al. 1996). This demonstrated that the panel was able to rank different cultivars consistently, and use an agreed vocabulary and scoring scale. The same panel was employed in the present study.

A range of physical measures has been developed in an attempt to reflect and standardise the sensory perceptions of fruit texture. Apple firmness has traditionally been measured as the maximum force required to push a manually operated Magness-Taylor (MT) fruit-firmness probe (penetrometer), of specified shape and with an 11-mm (in some market regions, 8 mm) diameter tip, 7.9 mm into peeled cortex tissue on opposite sides of the fruit equator (Magness and Taylor 1925; Bourne 1974; Lehman-Salada 1996). Force is applied perpendicular to the cut surface in a smooth motion in 2–3 s, with the depth of penetration in manual tests controlled by the operator. The MT measurement is accepted as the standard firmness measurement in the apple industry (Abbott 1994). The data obtained are expressed in terms of the force required to rupture cortex parenchyma cells, and thus represent a compound of many cellular and macrocellular properties including cell turgor and wall strength. Many variables affect the reliability of destructive firmness measurements. Lower readings are associated with slow insertion speed, shallow probe penetration, or with apples that are large, water-cored, bruised or warm (Blanpied et al. 1978). Due to the relatively simplicity of obtaining data with hand-held MT penetrometers, readings are also used as a measure of maturity and ripeness in commercial production.

Techniques for the non-destructive measurement of apple texture have been developed using sonic or vibrational methods (Abbott et al. 1992; Chen and De Baerdemaeker 1993). The indices these detect are essentially the (coefficient of elasticity of stretching), or the stiffness of intact fruit. Stiffness may thus be assessed in a non-destructive manner by measuring the acoustic resonance of fruit which has been struck with a light object (Chen and de Baerdemaeker 1993).

To date there have been relatively few studies relating to the genetic basis of the firmness of apple, or indeed of any fruit. Genetic linkage maps for *Malus* are now available (Hemmat et al. 1995; Maliepaard et al. 1998). The European Apple Linkage Map (Maliepaard et al. 1998) was constructed from segregation data of 290 markers scored on 152 individuals from a replicated reference mapping population. This population derives from a cross between the cultivars ‘Prima’ and ‘Fiesta’.

Data sets were accumulated over 2 years from six different sites, in order to maximise the possibility of detecting genotype × environment interactions. The individuals were grown at six sites in five countries, covering 13 degrees of latitude (King 1996). The experimental design allowed comparison of two different physical methodologies, comparison of site effects, and comparison of physical measurements with sensory perceptions. Quantitative trait analyses were carried out to study the genetic basis of fruit firmness in apple, by identifying loci in the context of the existing linkage map.

Materials and methods

Plant material

A cross between ‘Prima’and ‘Fiesta’ was carried out at CPRO-DLO, Wageningen, in 1988, using ‘Prima’as the female parent. Unselected seedlings were raised in pots, and planted in the nursery at Wageningen in the winter of 1990/1991, and later planted on their own roots at Elst. Six replicate trees of each genotype were obtained by budgrafting wood of 152 seedlings onto M27 dwarfing rootstock in early 1992. The trees were grown for another year. One set was grown on rootstock at Elst and the remainder were distributed to the five additional sites in early 1993 (Table 1). At Cadriano only one-half of the population was grown.

The trees were grown either in rows 2 m apart or at East Malling in cordons 1 m apart. Up to five replicate trees of the parents were planted at each site, but these replicate trees were often not assessed separately. The relative positions of the trees were randomised beween Elst, Naoussa, East Malling and Cadriano. At the remaining sites, the trees were in the same order as the original seedlings at Elst. Normal cultural practices were followed. Trees were sprayed with insecticides and fungicides during the years that they were being evaluated for fruit characters, to prevent fruit quality being affected by pests and diseases.

Harvesting and sampling

Fruit were harvested up to twice a week as they reached the tree-ripe stage in 1995 and 1996 (Table 2). Fruit which were damaged by 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 fruit. 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 wrapped individually in tissue paper. Where the apples were not assessed at the site where they were harvested, they were stored at 4°C for up to 3 days, and transported by air- or land-courier as required.

Sampling for fruit firmness measurements

For penetrometer measurements, fruit from Wellesbourne were assessed at East Malling. For other sites, fruit were assessed locally.

Table 1 Locations of field sites for harvesting fruit from the replicated progeny of the 'Prima' × 'Fiesta' family

Site	Country	Location		Propagation
		Grid reference	Altitude (m)	
Wellesbourne	England	52° 12′N 1° 36′W	49	M27 staked
East Malling	England	51° 17′N 0° 27′E	32	M27 cordons
Elst	The Netherlands	51° 55′N 05° 50′E	8	M27 staked, own roots
Angers	France	47° 30′N 00° 35′W	57	M27 staked
Cadriano	Italy	44° 32′N 11° 23′E	30	M27 staked
Naoussa	Greece	40° 37′N 22° 07′E	121	M27 staked

Table 2 Methods used to assess fruit firmness and texture. Sensory measuresdescriptors of first-bite texture and texture during chewing were scored on a scale 0–100 based on an arbitrary scale of nil to extreme

Class	Descriptor		Units
Firmness	FFF-1	Fruit flesh firmness by penetrometer with 8–mm probe; 3 readings	kg
	FFF-2	Fruit flesh firmness by penetrometer with 11-mm probe; 20 fruit	g
Stiffness	FST-RES-1	Fruit stiffness determined by acoustic resonance	$Hz^2.g^{2/3}$
First-bite texture	Hardness	Take one bite from the segment with front teeth	Nil to extreme
Texture during chewing	Crispness	How crisp the apple seems during chewing – hard but brittle, makes a characteristic crunchy noise when chewing.	Nil to extreme
	Granularity	Disintegrates into small granules when fruit is chewed	Nil to extreme
	Spongy texture	Pulpy/fluffy type of texture	Nil to extreme
	Slow breakdown	Speed of breakdown of apple flesh (ignoring skin) in the mouth until state ready for swallowing	Nil to extreme
	Juiciness	Amount of juice produced during chewing	Nil to extreme
	Overall liking	How much the apple was liked overall	Nil to extreme

Sampling for stiffness measurements

For acoustic resonance measurements in 1995 fruit from CPRO, the Netherlands, were collected from the trees on their own roots and sent for assessment in Leuven. In the 1996 trial, fruit-set data across all sites were collated in June and a sampling strategy devised. Due to limitations on sample-throughput for the acoustic resonance measurements, we maximised population coverage from two sites (Wellesbourne and Angers, Table 3). To obtain data indicative of the genotype × environment (G × E) effect, eight fruit from up to ten individuals replicated from the six site/year combinations were selected.

Sampling for Sensory Evaluation

For sensory evaluation, fruit from East Malling and Wellesbourne were sent to Sensory Dimensions, Reading, England. In 1995 two batches of fruit, and in 1996 eight batches of fruit, were sent. Each batch consisted of 16 separate genotypes per batch included reference population segregants and fruit of both parent cultivars. The different batches were assessed separately.

Trait assessments

Penetrometer readings

A small area (up to 15 mm diameter) of peel was removed from each apple fruit with a knife. The fruit was then placed on a stand, and the resistance of the flesh determined using a mechanised 8-mm MT probe (FFF-1, Table 2). At Angers an automated electronic penetrometer (Penelaupe) was used with an 11-mm (FFF-2) MT probe. Resistance was expressed in g force. The arithmetic mean of readings from three separate fruit was recorded.

Acoustic resonance

The acoustic resonant frequency of fruit was determined using the Acoustic Response Technique (Chen and De Baerdemaeker 1993) and recorded in Hz; the mass of each fruit was determined and the stiffness calculated from Stiffness = (Resonant Frequency corrected for size/weight).[$Hz^2.g^{2/3}$]. Readings were taken on receipt of the fruit (day 0) and at 7, 14 and 21 days during storage at 20°C, to reflect shelf-life changes which would occur during transport or retailing of the fruit.

Sensory analysis

The fruit were evaluated by a trained sensory panel of 11 individuals which had been used in the 2 previous years. As part of an initial standardisation process the panelists scored a comprehensive range of attributes on fruit from 12 commercial varieties. These scores were subjected to principal component analysis, as well as analysis of variance and a non-parametric test of rank interaction. Following this, a non-redundant set of descriptor terms was selected such that each descriptor showed significant differences between the varieties. Analysis of the panelist × variety interactions showed that although interactions were present for most of the descriptors, the interactions never affected rank order (Daillant-Spinnler et al. 1996). It was therefore concluded that the mean scores for each variety given by the panelists for each descriptor could be considered satisfactory estimates of the sensory profiles of the varieties.

Table 3 Assessments of fruit texture carried out on the replicated progeny of the cross between 'Prima'× 'Fiesta' at different sites and years. Fruit for FST-RES-1 were transported to Leuven, Belgium, for assessment. Fruit for sensory measures were sent to Reading, UK, for assessment. Note that the numbers of fruit per genotype given are the largest in a sample. Some genotypes yielded fewer fruits than this

[a] Together with a further five fruit per genotype from 11 genotypes
[b] Together with a further four fruit per genotype from ten genotypes
[c] Eleven panel members assessed each genotype, using sectors from one fruit or more

Descriptor	Site	Fruits per genotype	Year	Genotypes sampled
FFF-1	East Malling	3	1995	37
	East Malling	3	1996	105
	Wellesbourne	3	1995	35
	Wellesbourne	6	1996	108
	Elst	3	1996	129
	Cadriano	3	1995	43
	Cadriano	3	1996	82
	Naoussa	3	1995	81
	Naoussa	3	1996	145
FFF-2	Angers	20	1996	74
FST-RES-1	Wellesbourne	7[a]	1996	106
	Elst	25	1995	75
	Elst	12	1996	8
	Cadriano	12	1996	12
	Naoussa	12	1996	10
	Angers	8[b]	1996	112
Sensory descriptors	East Malling	11[c]	1996	49
	Wellesbourne	11[c]	1995	27
	Wellesbourne	11[c]	1996	63

Each fruit was peeled and then assessed for a number of attributes relating to internal odour, first-bite texture, internal appearance, texture and flavour during chewing. Descriptors for first bite-texture and texture during chewing were included in this study, along with an overall liking score (Table 2).

In 1995, the samples included 'Prima', 'Fiesta' and 27 segregant genotypes harvested from Wellesbourne. In 1996 the samples included 'Prima', 'Fiesta' and a total of 105 genotypes harvested from East Malling or Wellesbourne, of which seven were sampled from both sites. The total number of genotypes sampled was 115. Each descriptor was scored individually by 11 panel members on a scale of 0–100, denoting nil to extreme. The mean of these 11 scores was then taken. Due to the limited sample availability, one tasting of each sample was made by each assessor. Fruit from individual replicate trees of each parent, 'Prima'and 'Fiesta', were kept separate.

Statistical analysis

The trait data were analysed using REML (Patterson and Thompson 1971) in the statistical package Genstat 5 (Payne et al. 1993). REML is an analysis of variance also suitable for unbalanced designs. Data were analysed first with all factors as random to obtain the variance components. Estimates of the proportions of the variance contributed by 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 referred to as heritability). In order also to obtain the estimates of genotype means for QTL analysis, the analysis was repeated, first with the genotype as fixed to obtain the overall genotype estimates, then with both genotype and genotype × environment fixed, to obtain the genotype estimates per environment. Data were weighted according to how many fruit of a tree had been scored.

Penetrometer data were analysed with crossed effects of genotype and environment and an individual-tree effect nested within this (although the only trees replicated on any site were the parents). The environment effect was represented by a single factor having a different level for different sites or years. In the analysis of the penetrometer data, only datasets scored using the descriptor FFF-1 were analysed (Table 2). Attempts to include datasets scored using FFF-2 resulted in substantially increased error estimates, even after scaling.

The analysis of stiffness data from acoustic resonance measurements was broadly similar. A preliminary analysis considered the effect of time in store as randomised, and indicated that although there was a substantial linear trend of stiffness with time in store, there were no apparent effects of interest, such as interactions with genotype (data not presented). Consequently, in order to avoid the difficulties of a repeated measures analysis on these unbalanced data, the formal analysis was confined to the data from acoustic resonance measurements prior to storage.

For the analysis of the sensory data, the effects included were those of the batch of fruit, genotype with the site nested within it, and a residual term.

The overall genotype estimates of the penetrometer readings, stiffness and the sensory descriptors were then analysed by principal component analysis. Due to the differences in scale, correlations rather than variances were analysed. The components accounting for most of the variance were subjected to QTL analysis.

QTL analysis

QTL analysis of the REML estimates for genotype, per environment and over environments, was performed using the Maximum Likelihood-based interval mapping approach of MapQTL ver. 3.0 software (Van Ooijen and Maliepaard 1996). This version of the program enables QTL analysis of a full-sib family of a cross-pollinating species with four QTL alleles per segregating QTL. The integrated linkage map of 'Prima'and 'Fiesta' was used (Maliepaard et al. 1998). This map consisted of 290 markers (124 RFLPs, 133 RAPDs, 10 SSRs, 17 isozymes, four AFLPs, one CAPS-RFLP and one SCAR). Of these markers 127 were 'Prima' markers, 96 were 'Fiesta' markers and 67 markers were heterozygous in both parents. Of the heterozygous markers 49 segregated for three or four alleles and thus were fully informative, while the remaining 18 segregated for two alleles. Linkage phases between markers were known from linkage analysis. Since the integrated linkage map consists of markers with different segregation types, the all-markers mapping approach (Knott and Haley 1992, Maliepaard and Van Ooijen 1994) was used to upgrade marker information. In this method markers from neighbouring intervals, as well as flanking markers, are used to calculate the probabilities of QTL alleles. Five neighbouring intervals were employed, except for linkage group L1, where ten neighbouring intervals were used. This was done as the bottom end of L1 was covered mostly by markers which were informative for 'Prima'alleles only. By employing ten neighbouring intervals, information from 'Fiesta' alleles could also be included. A 1-cM step size was used.

For interval mapping, a LOD score threshold of 3.0 was employed to indicate evidence for a QTL (suggestive linkage). 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, personal communication). 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 selected sensory data, using this feature in MapQTL (Van Ooijen and Maliepaard 1996).

Results

Trait distributions

The distribution of trait values across the population differed between environments and occasions, with evidence of transgressive segregation in all cases. All measures indicated that fruit of 'Prima'were less firm than 'Fiesta'. 'Fiesta' (Fig. 1a, b, c). For penetrometer readings and stiffness measured by acoustic resonance, there were differences in the amount and direction of skewness in the distributions.

Variance components

For the penetrometer data, 43% of the variability is accounted for by the site/year (= environment) combination. A further 25% is accounted for by the genotype × environment interactions, with a smaller (20%) though still highly significant ($p < 0.001$) effect of genotype. For the stiffness data, the variability accounted for by the genotype was 14%, and that for genotype × environment was 9%.

The genotype × environment interaction was not significant ($p = 0.05$) for any of the sensory descriptors. The effect of the batch of fruit was significant ($p < 0.05$) only for slow breakdown and hardness.

Heritability estimates for the sensory traits ranged from 14% (granularity) to 57% (crispness). In decreasing order the estimates were: hardness (52%), slow breakdown (51%), sponginess (48%), juciness (46%) and overall liking (28%).

Relationships between measurements

A scatter-plot matrix of the different measures is shown in Fig. 2. The penetrometer measurement FFF-1 has a

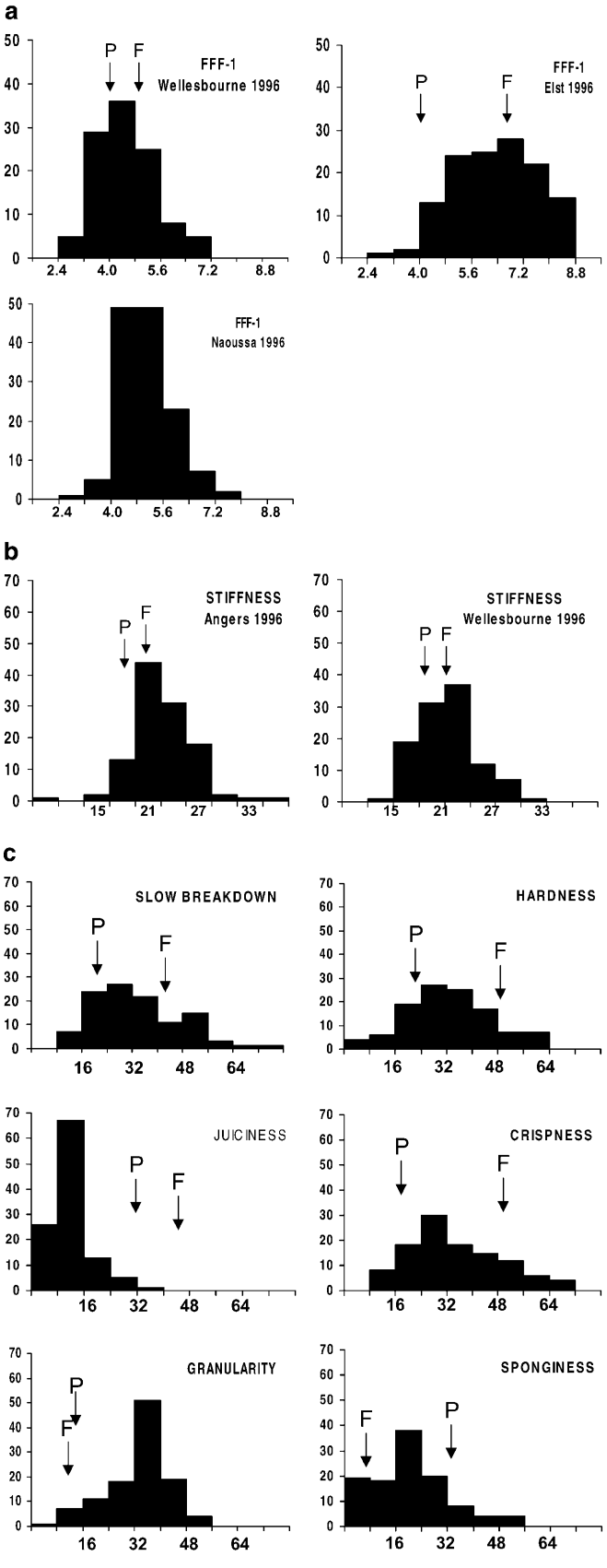

Fig. 1a–c Histograms of genotype estimates for traits relating to fruit texture scored on individuals from the segregating population derived from 'Prima' × 'Fiesta'. Values were calculated using REML. Y axis = number of genotypes in class. Mean parental values are indicated with *arrows*: '*P*' = 'Prima'; '*F*' = 'Fiesta'. **a** Histogram of fruit firmness scores recorded in 1996. **b** Stiffness as measured by resonant frequency in 1996. **c** Sensory measures assessed in 1996

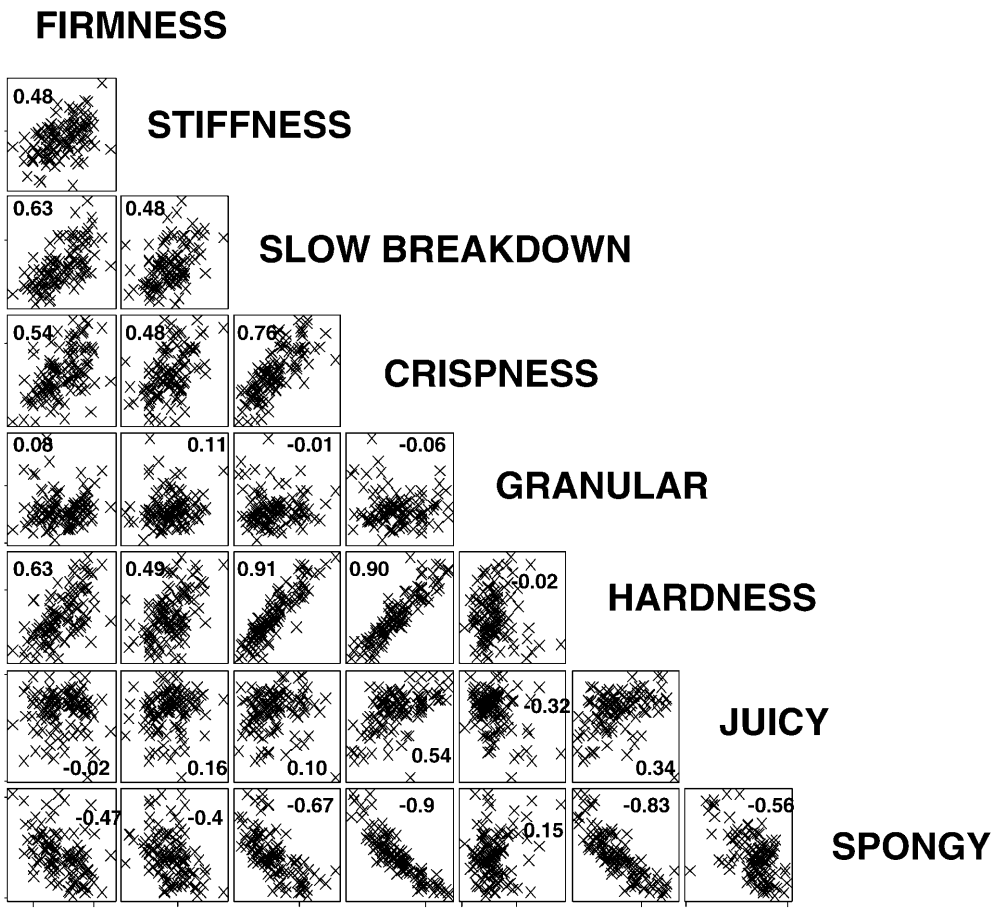

Fig. 2 Scatter plot matrix of mechanical and sensory measures.descriptors. Correlation values have been added to the plots. Individual points correspond to genotype estimates using REML

correlation of 0.63 with the sensory measuresdescriptors of hardness and slow breakdown, and of 0.54 with crispness. The stiffness descriptor is not correlated very highly with any of the other measurements, having a correlation of just below 0.5 with the penetrometer value, slow breakdown, crispness and hardness. Among the sensory descriptors, hardness has a correlation over 0.9 with slow breakdown and crispness, although that between crispness and slow breakdown is only 0.76. Crispness is correlated negatively (– 0.9) with sponginess. Juiciness has a correlation of 0.54 with crispness, and is also correlated positively with hardness, and negatively with sponginess (– 0.83) and granularity.

The first principal component accounts for 55% of the total variability. It appears to represent an overall firmness measure as it comprises approximately equal weights of hardness, crispness, negative sponginess and slow breakdown together with penetrometer readings and stiffness, and a smaller component of juiciness. The second principal component accounts for a further 19% of the variability, and consists mostly of a contrast between juiciness and granularity, with smaller components of crispness and negative sponginess associated with the juiciness, and of penetrometer reading and stiffness with the granularity. The third principal component, explaining just under 10% of the variability, consists of a contrast between juiciness, granularity, and a smaller component of stiffness with penetrometer reading and slow breakdown. We have used negative sponginess in this description since sponginess is generally of the opposite sense to the other measures (Fig. 2).

QTL analysis

QTL mapping results are presented in Table 4. For the penetrometer data (FFF-1), LOD scores greater than 3.0 in at least one environment were observed for 12 of the 17 linkage groups. A possible QTL on linkage group L01 is observed in five of the ten site/year combinations, in five out of the six sites, all of them in 1996. The LOD score for the sixth site (Angers) was 2.9. A possible QTL on linkage group L10 was observed also for five of the ten site/year combinations. Significant linkage (LOD score greater than 4.5) was observed on linkage groups L01, L06, L10 and L12 in different environments. Results of regression-based interval mapping confirmed those of the maximum-likelihood approach, the absolute LOD-score difference usually being less than 0.5. Only for L06 was the LOD score much smaller with regression than with the maximum-likelihood approach (LOD score of 3.7 compared to 4.8). For the overall estimates (over environments) linkage groups L01, L08 and L10 yielded LOD scores greater than 4.5.

Table 4 QTL associated with penetrometer readings, stiffness and sensory descriptors, detected with the interval mapping technique. 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 explained by the putative QTL indicated below. Kruskal-Wallis tests were carried out for 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 OPT-16–1000 on L16 as a co-factor, a significant QTL was detected on L01 for Juiciness. Additional suggestive QTL were detected as indicated (M^b). Ninf = number of individuals for which data for the quantitative trait were available

Descriptor	Site	Ninf	L01	L02	L03	L04	L05	L06	L08	L09	L10	L12	L13	L15	L16
FFF-1	Naoussa 1995	79									4.0				
	Naoussa 1996	132	3.0	3.1							4.9 *18%*			3.1	
	East Malling 1995	35										5.9 *60%*			
	East Malling 1996	101	3.3					4.8 *28%*	4.4		3.6				3.7
	Wellesbourne 1995	34			3.2								3.1	3.0	
	Wellesbourne 1996	106	3.7								7.1 *29%*				
	Elst 1996	125	4.6 *16%*			4.1			4.0	3.1	3.0	3.0			
	Cadriano 1995	42			3.1			3.0							3.2
	Cadriano 1996	79	4.1	3.4				3.9							
FFF-2	Angers 1995	73												4.0	
FFF	Overall	152	6.5 *19%*		3.6 *17%*				4.7 *16%*		7.4 *22%*			3.5 *10%*	
Stiffness	Wellesbourne 1996	104	3.7								4.6 *21%*				3.2
	Elst 1995	72	4.0												
	Angers 1996	111	3.4					3.1							
	All sites	144	4.4								4.5 *16%*				
Sensory	Slow breakdown	113	*				3.6 **		***		3.5 *	3.1		3.1	
	Crispness	113	3.5				M^b				3.2	3.9	M^b		6.0 *24%*
			*				**	*	**			*	*		****
	Granularity	113		5.1 *24%*					3.0				*		3.0
	Hardness	113					3.7 ***	3.4 **	***		3.7 *	*		3.2	3.8 ***
	Juiciness	113	3.6[a]									M^b			14.8 46%
	Sponginess	113	3.3				M^b	M^b						3.1	7.7 30%
	Overall-liking	113										M^b			11.3 *38%*
													*		****
PCP	1st Principal comp	110	3.4				3.2	3.1			4.2	3.4		3.4	5.1 *22%*
	2nd Princ. comp	110	4.0						3.0						7.8 *28%*
	3rd Princ. comp	110													

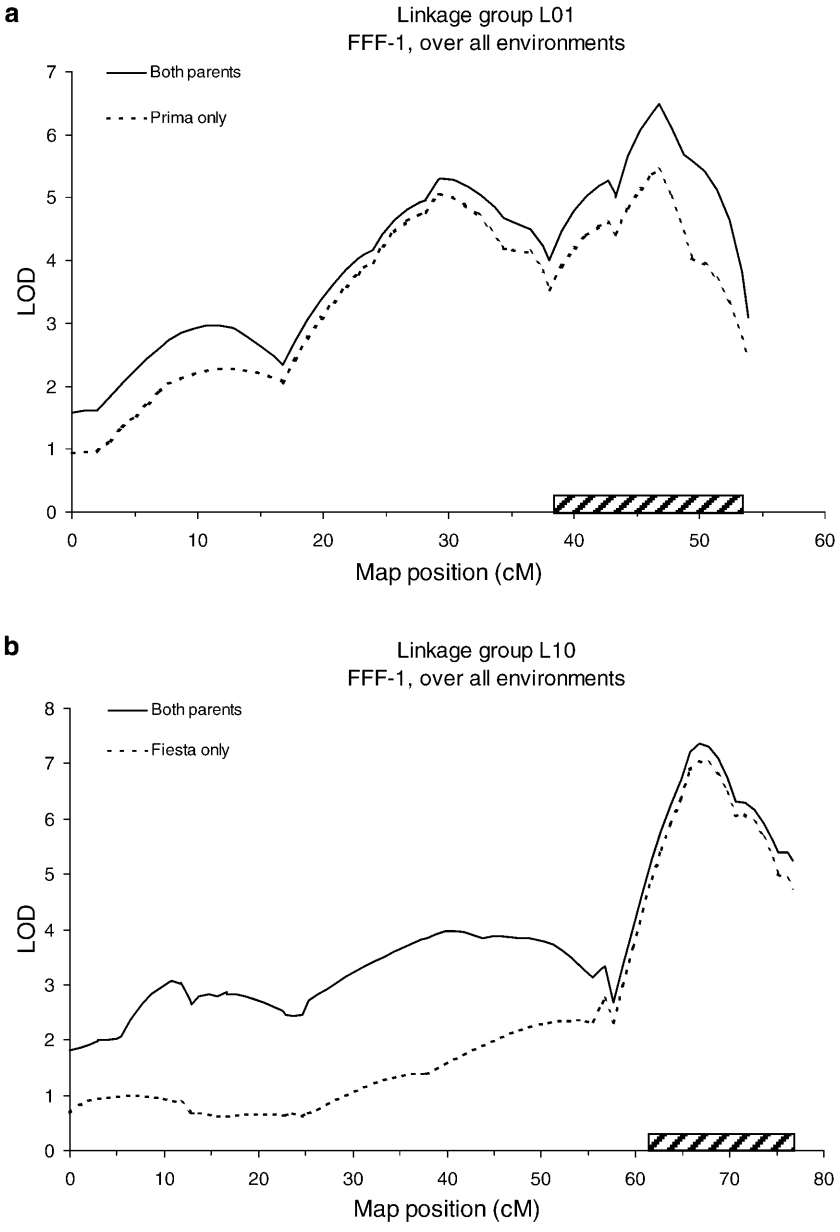

Fig. 3a, b LOD plots resulting from interval mapping of REML estimates of the FFF-1 trait data over all environments. Solid lines indicate results where marker data from both parents were used. **a** QTL detection on linkage group L01. The *dashed line* indicates results from using only the 'Prima'map and 'Prima'markers. **b** QTL detection on linkage group L10. The *dashed line* indicates results from using only the 'Fiesta' map and 'Fiesta' markers. Two-LOD support intervals for the map position of the QTLs are indicated by *bars along the x-axes with diagonal hatching*

For the resonant-frequency data, LOD scores greater than 3.0 were observed for four of the 17 linkage groups. LOD scores greater than 4.5 were observed for linkage group L10 only. A possible QTL on L01 for the combined data was just below the threshold of 4.5.

The QTL on linkage group L01 for the penetrometer and resonant-frequency data was primarily as a contrast between the two 'Prima'alleles. The QTL maps to a region introgressed from a wild apple species, *Malus floribunda* 821 and which includes the *Vf* gene, which confers scab resistance (King et al. 1998). The QTL allele which contributes to firmer fruit is in coupling phase with *Vf*. The penetrometer overall mean was analysed again, using only the 'Prima'map and 'Prima'markers to verify whether the QTL effect was due to a 'Prima'allele. The results of both analyses are presented in Fig. 3a. The QTL from linkage group L10 was mostly a contrast between the 'Fiesta' alleles. Here also the data were re-analysed using only 'Fiesta' markers and the 'Fiesta' map to verify this (Fig. 3b). The differences between the LOD graphs are small, indicating that in each case the QTL is an effect of a single allele from a single parent, i.e. a contrast between the 'Prima'alleles in the case of L01, and a contrast between the 'Fiesta' alleles in the case of L10.

For the sensory data sets, LOD scores greater than than or equal to 3.0 were found for nine linkage groups for one or more of the sensory descriptors. LOD scores greater than 4.5 were observed for L02 and L08, and for L16 for three of the sensory descriptors. The high LOD score for L02 for granularity was not confirmed by regression. Linkage groups L01 and L10 showed LOD scores greater than 3.0 for slow breakdown and hardness (L10 only), crispness (both), juiciness and sponginess

(L01 only). Although less clear, here also the QTL on L01 seem to be mainly the effect of one 'Prima'allele in coupling phase with *Vf*. For juiciness, one allelic combination of the QTL seemed to give less-juicy fruits than the other three allelic combinations. The QTL on L16 gave high LOD scores for crispness, juiciness, sponginess and overall liking. This QTL was mapped to the region of *Ma*, the malic acid gene (Maliepaard et al. 1998). The effect was a combined effect of alleles from both parents, showing a distinct contrast between individuals in the progeny with the dominant *Ma* and the recessive *ma* phenotype, the *Ma* phenotype (more acidic) being the more favoured in all three cases.

For crispness, juiciness, sponginess and overall liking, a marker closely linked to *Ma* was used as a cofactor in MQM analysis, so that this marker (OPT-16–1000) would absorb variation due to the QTL in this region. For crispness, possible QTLs (LOD > 3.0) were observed on L05 and L013, in addition to those previously observed on L01, L10, L12 and L16 (Table 4). For juiciness, a possible QTL was observed on L12, and significant linkage (LOD score > 4.5) was now observed for the QTL on L01. For sponginess, possible QTLs were observed on L5 and L6, and for overall liking, on L12.

In the QTL analysis of the first two principal components, LOD scores greater than 4.5 were found for L16, while LOD scores greater than 3.0 were found for L01, L05, L06, L08, L10, L11 and L15. No LOD scores greater than 3.0 were found for the third component.

The 2-LOD support intervals were calculated for the QTL for the overall mean of the penetrometer data, for linkage groups L01, L08 and L10. Simulation studies have shown that a 2-LOD support interval usually has a probability over 95% of containing the QTL (Van Ooijen 1992). The 2-LOD support interval for L01 was [39.0–53.4 cM], for L08 the 2-LOD support interval was the interval [32.1–53.5 cM] and for L10 the interval [61.7–76.2 cM]. The 2-LOD support intervals for L01 and L10 are indicated in Fig. 3.

Discussion

The trait distributions for penetrometer readings, stiffness and some of the sensory descriptors show transgression of the progeny towards softer fruit and firmer fruit. In all cases 'Prima' had softer fruit than 'Fiesta', and in all sensory descriptors 'Fiesta' was favoured over 'Prima'. The measures juiciness and granularity had skewed distributions, with the parents clustered towards one end. This may reflect strong selection for juiciness in marketed cultivars.

Large environmental effects and large genotype × environment interactions were observed for physical measurements of fruit firmness. These effects complicate the selection in one environment for performance in different environments, if traditional breeding methods are used. Rather small heritability estimates were obtained for penetrometer readings and stiffness as measured with acoustic resonance, with those for stiffness being smaller than for penetrometer readings. Despite these high genotype × environment interactions and low heritabilities, a number of QTL for fruit firmness characters were detected in this study. Apparently some of these were effective in multiple environments, and perhaps even in those environments where the LOD score in this study did not exceed the significance threshold. Indeed some other elevated LOD scores for linkage groups L01 and L10 were observed, but these remained below the thresholds, due perhaps to larger environmental bias or smaller population size. This indicates that marker-assisted selection, at least for these main QTL, may be feasible and effective for multiple environments, whereas the effectiveness of traditional selection methods without the additional information provided by markers might be hampered by environmental noise and interaction effects.

There are concerns about the relevance or reliability of penetrometer tests since they are not always considered to reflect accurately the textural components "crisp", "tough" or "mealy" (Lurie and Nussinovitch 1996). In the current study, low to moderate correlations were found between physical measurements of fruit firmness and between physical and sensory measurements. These correlations were determined across 147 different genotypes. Although Lurie and Nussinovitch (1996) had found much higher correlations for penetrometer and sensory descriptors, their data were from only two cultivars, and correlations were within cultivars. Our observations confirm recent findings based on inter-varietal comparisons (Barreiro et al. 1998).

Correlations among sensory descriptors were high for hardness, crispness, slow breakdown and negative sponginess. Correlations of these descriptors with juiciness were smaller, and granularity showed only very small correlations with the other sensory descriptors. The higher correlations may be attributed to perceptual interactions. The relationship between crispness and juiciness is likely to arise from cortex tissue of very crisp apple fruit possessing stronger inter-cellular bonds in the middle lamella of adjacent cell walls. When such fruit is bitten, the cells are more likely to fracture and release juice. A weaker middle lamella leads to fracture between cells and thus gives rise to the perception of a mealy or granular texture. This is reflected in the negative correlation of crispness and juiciness with granularity. It is also possible that the detection of juiciness is emphasised by the co-segregation of acidity, which also contributes to an overall-liking descriptor which was scored by the same panel. However, recent studies based on three commercial cultivars, each presumably of normal acidity, have also shown a high correlation between crispness and juiciness (Barreiro et al. 1998). They also observed higher correlations of these descriptors with instrumental measurements, these being the confined compression of fruit cylinders and acoustic impulse response.

Despite the moderate correlations between penetrometer readings, stiffness and sensory data, there was evidence for QTL for each of these on linkage groups L01

and L10. In the sensory evaluation, L01 and L10 show up for those data which are expected to reflect firmness most strongly: crispness, hardness and slow breakdown. More QTL were detected for penetrometer readings than for stiffness or individual sensory descriptors. This may be explained by a higher number of replicate observations of the genotypes and the higher estimated heritability for penetrometer readings. Also, the possibility cannot be excluded that the penetrometer reading comprises more and different underlying genetic components than those involved in stiffness and sensory descriptors. As a consequence of the lower heritability estimate and the somewhat lower correlations with sensory descriptors, the value of stiffness measured by acoustic resonance may be rather limited in breeding selection. For the breeder, the ease of determination of fruit firmness with a penetrometer is also likely to continue to favour use of the latter.

The two most significant QTL for penetrometer readings were shown to be derived from the respective parents. The QTL on L01 appeared to be a contrast of the 'Prima' alleles, with no significant effect from the 'Fiesta' alleles (Fig. 3a) whereas with the QTL on L10 the effects were reversed. This demonstrates that each parent possesses different QTL alleles for penetrometer readings, and that even 'Prima', which has softer fruit than 'Fiesta', is able to contribute to increased fruit firmness. This is in agreement with previous findings from breeding studies. It also emphasises the value of carrying out QTL analysis simultaneously for both parents in a cross. For example, a QTL analysis which was based on one parent expected to contribute to greater fruit firmness would overlook possible QTL contrasts segregating from the other parent. A similar situation has been exploited in tomato, where advanced backcross QTL analysis has been able to detect 25 alleles from a wild relative which improve traits from a horticultural perspective, despite the fact that overall the donor is phenotypically inferior to the elite parent (Bernacchi et al. 1998). It is also in agreement with the distributions shown in Fig. 1a and b which illustrate transgressive segregation in the progeny, both towards firmer fruit and towards softer fruit.

The QTL identified on linkage group L01 of 'Prima' is linked to the introgressed region originating from *Malus floribunda* 821 carrying the *Vf* locus which confers scab resistance (King et al. 1998). The allele contributing to firmer fruit is in coupling phase with *Vf*. Although there was a small gap in between two LOD peaks on linkage group L01, there was no evidence for a second QTL on this linkage group. This was verified by using a marker as a cofactor on this linkage group and testing for the presence of another QTL. It was also confirmed in Bayesian analysis (Maliepaard and Sillanpää, in preparation).

The sensory analysis attempted to address the ability of different human perceptions to resolve variation in aspects of apple-fruit texture. The use of a trained panel to assess large populations of fruit in a genetic analysis is rare, and appears to have been successful. We have been able to demonstrate that such data may be used to detect genetic effects. The fact that relatively few QTL were detected for the sensory descriptors may indicate that these do not reflect very strongly the underlying genetic factors, perhaps due to large environmental effect, or due to masking of these genetic factors by other genes which may overrule their expression in human perception. However, it is also possible that the sensory descriptors represent simple traits. The crispness QTL on group L16 has a high LOD score (6.0), and accounts for 17% of the variance. For sponginess, a LOD score of 7.7 was obtained in this region and this QTL accounts for 30% of the variance. QTL for hardness and granularity are also suggested in the same region. These sensory QTL map to a region known to contain the acidity locus *Ma* (Maliepaard et al. 1998). Three aspects emphasise the correspondence of these sensory QTL with the *Ma* locus. Firstly, they are apparently linked rather closely, as shown by the LOD peaks at the *Ma* locus. Secondly, the segregation of the sensory QTL appears to be identical to the segregation at the *Ma* locus: of the four possible QTL genotypes for each sensory descriptor, the estimated mean of one QTL genotype is in the direction of less firm, less juicy and more spongy fruit, whereas the other three QTL genotype means are approximately equal. In each case, the less-favoured QTL genotype corresponds to the *mama* genotype in the progeny. This suggests dominance of a QTL allele present in both parents, identical to the situation of *Ma*. Finally, this favoured and dominant QTL allele is in coupling phase with *Ma*.

This association of the sensory texture QTL on L16 with the *Ma* locus may be due to perceptual interactions. The marked lack of taste of apples from *mama* genotypes may override other positive perceptions, whereas the better-tasting fruits from *MaMa* and *Mama* genotypes may mask some other negative attributes. The co-segregation with the *Mama* locus may also indicate that they are pleiotropic effects of a gene at the *Ma* locus. Finally, a cluster of different genes co-localised in the same region may exist. In peach, QTL for several fruit quality traits including acidity and soluble-solid content, measured instrumentally, have been located recently in the same regions of just two linkage groups (Dirlewanger et al. 1999). In tomato a region on chromosome 6 contained a QTL for fruit mass, pH and soluble-solid concentration (Paterson et al. 1991).

The experimental design was developed when little initial information about site-to-site variation existed for the particular measures. These and other results from the same research programme (King 1996; King et al. 1998) indicate that a modified design may be more appropriate in future. For accurate positioning of QTL it is important, within a given size of experiment, to maximise the number of recombinants whilst providing inter- and intra-site replication of only a relatively small proportion of the population in order to obtain accurate estimates of variance for site-to-site and occasion-to-occasion variation (Lynn 1998).

The identification of chromosomal regions contributing to major attributes of fruit texture is the first stage in devel-

oping selectable markers for the early selection of desirable genotypes. Several issues are raised. One relates to the accuracy of QTL position and the ability to predict effects in subsequent generations. The accuracy of mapped QTLs is still problematic. Some improvement can be made by using marker cofactors (Jansen 1993; Zeng 1994); further improvement can be made by using only fully informative markers, larger progenies or more generations, and by reducing missing values for markers and trait. In apple, where further pedigree testing is difficult or impossible, there are no major remedies. However, for marker–assisted early selection, it may be enough to increase considerably the probability of selecting the favourable genotype and this is feasible even for a QTL whose position is known with little accuracy. This becomes more problematic when there are disadvantageous genes linked to the positive QTL allele. The second issue relates to the conservation of the position of functional alleles in different genetic backgrounds. The results from these experiments would need to be validated in crosses or pedigree analysis involving cultivars other than 'Prima'and 'Fiesta' before universal statements could be applied.

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