Plant Breeding **131,** 322—328 (2012)
doi:10.1111/j.1439-0523.2011.01932.x

Mode of inheritance in fruit acidity in apple analysed with a mixed model of a major gene and polygenes using large complex pedigree

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With 5 figures and 2 tables

Received April 28, 2011/Accepted November 2, 2011
Communicated by W. E. Weber

Abstract

Fruit acidity is an important characteristic to determine the marketability of apple (*Malus* × *domestica* Borkh.). To reveal the mode of inheritance in fruit acidity and to estimate the genetic parameters, we performed segregation analysis using a population from an apple breeding programme. Four models (mixed, Mendelian, polygenic and environmental) were compared to find the most likely mode of the inheritance of acidity. The phenotypic variance of acidity in the population was properly explained using a mixed model of a major gene and polygenes. The genotypic values of the major gene (*AA*, *Aa* and *aa*) were estimated to be 0.45, 0.52 and 0.92 g/100 ml in titratable acidity, respectively. The values of the homozygote (*AA*) and the heterozygote (*Aa*) were very close and lower than that of the other homozygote (*aa*), indicating that an allele of the major gene appeared to have complete dominance with a function of lowering acidity. The estimate of the heritability after accounting for the major gene was moderately high, 0.43, in the mixed model. This means that even with removal of the effect of the major gene, acidity could fluctuate considerably by the effect of polygenes. The proportions of progeny with adequate acidity differed greatly, depending on which genotype was used as parents for the crossing. Therefore, it is very important to know the genotypes of parental cultivars before crossing.

Key words: genotype — heritability — *Malus domestica* — phenotype — titratable acidity

Apples are produced in much of the world and consumed fresh and as juice and processed products. Apples can be stored up to a full year, which supports their mass consumption in the world market. One important factor that determines the suitability of a cultivar for dessert or processing purposes is the acidity of the fruit. Because low-acidity apples have a less desirable aroma and insipid taste, particularly in processed products, high-acidity apples are essential for cooking, and low-acidity apples are used predominantly for desserts. Acidity also determines whether fruit should be consumed immediately or not and how long fruit can be stored because acidity gradually decreases during storage and an excessive decrease in acidity reduces the eating quality of the fruit. Therefore, acidity is an important characteristic determining the marketability of apples. Consumer acceptability of apples in Europe is closely correlated with the acid content (Kingston 1992).

Acidity is a characteristic that has been found to segregate within seedlings, according to early genetic studies of apple. The acid in a mature apple is almost entirely malic acid. The distribution of seedlings in the malic acid concentration showed two types of distribution among progeny. One was a simple distribution with one peak, while the other had two peaks: one below 0.3% (or pH > 3.8) and the other with a higher range of acidity (Nybom 1959, Visser et al. 1968, Brown and Harvey 1971). Because low acidity behaved as a recessive characteristic, medium-to-high acidity was considered to be dominant, and acidity was assumed to be controlled by a major gene (*Ma*). Moreover, a simple distribution was observed around the peak within a range of medium-to-high acidity, and polygenes were also suggested to control the malic acid concentration. Acidity in these early studies was expressed as the malic acid concentration determined by chromatography or as the pH. In modern apple breeding programmes, however, the acidity of fruit is measured by titration [titratable acidity (TA)]. Therefore, we cannot refer to the results of the early studies and take advantage of the results in our own breeding programmes.

Genetic research on quantitative traits has been restricted in studies on fruit because it is difficult to use trees as experimental plants. Trees generally require several years after planting to bear fruit and occupy a large amount of space. Study on the inheritance of acidity in apples has not advanced since the early reports. The inheritance of acidity in apple seems to be explained by three components: the effect of a major gene of large effect, the residual additive heritable effects of polygenic loci and the independent random effects of the environment. This is commonly referred to as a mixed model. The most powerful test for the mixed model is a segregation analysis, which uses information of pedigree to specify the underlying distributions of phenotypic values (reviewed by Lynch and Walsh 1998). When a major gene is segregating, the phenotypic distribution can exhibit multimodality, skewness and/or kurtosis. The segregation analysis is a useful method because only phenotypic records from a pedigreed population are required. The objectives of this study were to reveal the mode of inheritance in fruit acidity in apple and to estimate the genetic parameters by segregation analysis.

Materials and Methods

Progeny population: This study was carried out using a population from the apple breeding programme at the National Institute of Fruit Tree Science (NIFTS), Morioka, Japan. The population consisted of

16 cultivars as parents and 408 individuals from 31 families, with one to 48 unselected individuals per family (Fig. 1). Crosses were made solely for producing commercial cultivars and therefore had no specific mating design. As a result, the frequency of use of a cultivar in crosses differed greatly among parental cultivars. The pedigree structure of the 16 parental cultivars is shown in Fig. 2. The first line of the pedigree structure indicates the founders. Four of the founders ('Delicious', 'Ralls Janet', 'Jonathan' and 'Golden Delicious') originated in the USA; two of the founders ('Cox's Orange Pippin' and 'Worcester Pearmain') are from England, and the remainder, 'Indo', is from Japan. The other cultivars, except for 'Kidd's Orange' and 'Gala', were produced from public and private apple breeding programmes in Japan. Progenies were grafted onto dwarfing rootstocks and planted in 2000–2002 in an orchard of NIFTS. Trees within a family were planted

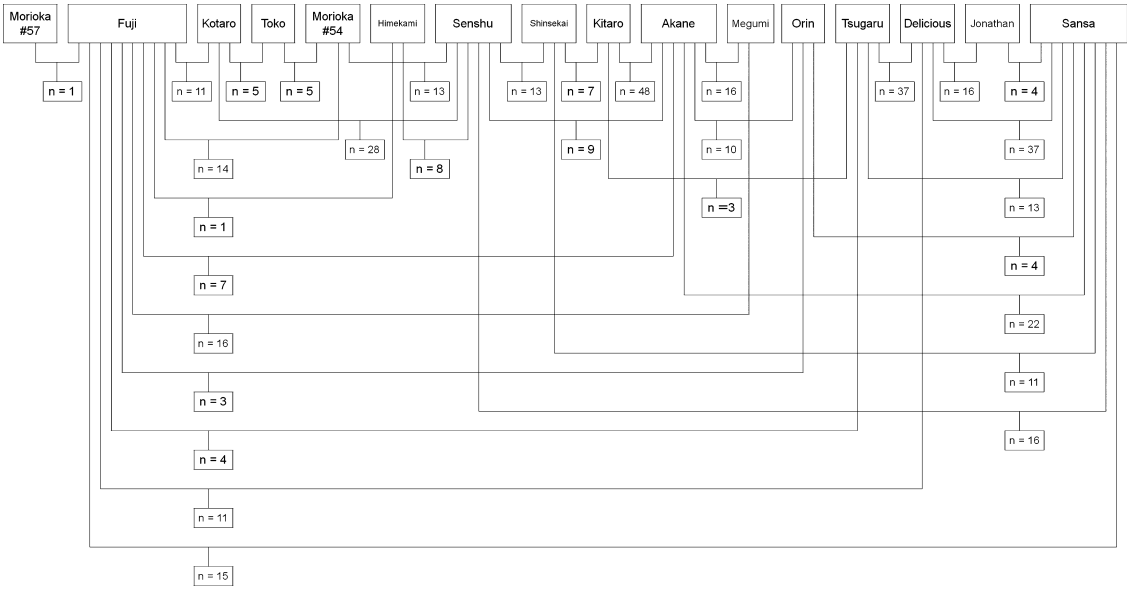

Fig. 1: Pedigree structure of progeny used in segregation analysis. The first line indicates parental cultivars. n is the number of progenies obtained from each cross

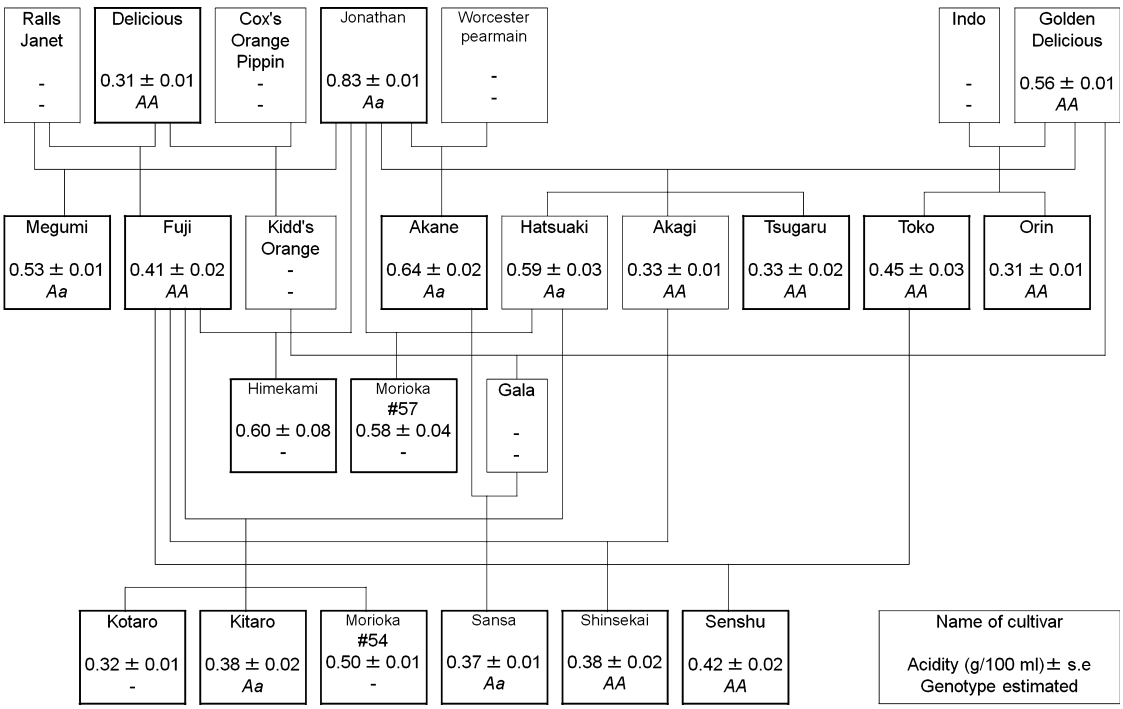

Fig. 2: Pedigree structure of parental cultivars used in segregation analysis. The names of the cultivars, acidity and genotype in acidity are enclosed in rectangles. Acidity is expressed as an average of 3 years of data. The genotype in acidity is estimated by one of the functions equipped in the pedigree analysis package (PAP). The genotype was determined when the probability of the estimation was >90%, computed by PAP. The dashes in the second and/or third lines of the rectangles indicate that acidity was not measured and the genotype was not accurately estimated. The cultivars in bold rectangles were used as parents in the population examined

in plots at a spacing of 0.5 m within a row with 3 m between rows. Parental plants were grown in adjacent fields.

Fruit materials and measurement of acidity: Three fruit samples were randomly harvested from a tree of each progeny when most of the fruit was determined to be mature by sensory evaluations based on ground colour, texture, flavour and starch staining in 2005. In parental cultivars, five fruits were sampled at commercial maturity from August to November 2003, 2004 and 2005, and the phenotypic values of each parental cultivar were expressed as an average of the 3-year data. Acidity was measured as the TA. Crude juice was extracted with a juicer (MJ-C68; National, Osaka, Japan) from each fruit. After filtration, 2 ml of juice was titrated with an autotitrator (AUT-301; TOA Electronics, Tokyo, Japan) to pH 8.0 with 0.1 N NaOH. Titration results are calculated as malic acid (in grams) per 100 ml of the sample juice.

Segregation analysis: According to Snow and Wijsman (1998), we compared four models that can be fit to observed pedigree data to find the most likely mode of inheritance of acidity:

Mixed model: $y_i = \mu + g_i + a_i + e_i$

Mendelian model: $y_i = \mu + g_i + e_i$

Polygenic model: $y_i = \mu + a_i + e_i$

Environmental model: $y_i = \mu + e_i$

Where y_i is the TA of ith progeny, μ is the population mean, g_i is the effect of the major gene of ith progeny (the offset from μ for the major-gene genotype of ith progeny), a_i is the polygenic component of ith progeny, and e_i is the random environmental component of ith progeny. For founders, the a_i is assumed to be distributed $(0, \sigma_a^2)$, where σ_a^2 is the additive genetic variance after removing the genetic variance contributed by the major gene. Non-founders are distributed $N(a_{\text{father}} + a_{\text{mother}}/2, \sigma_a^2/2)$. The e_i is distributed $N(0, \sigma_e^2)$. Heritability (h^2) was defined as $h^2 = \sigma_a^2/(\sigma_a^2 + \sigma_e^2)$, which is commonly used in the context of segregation analysis. First, the observed pedigree data were analysed with the mixed model, and the frequency of the major gene and genotypes of the major gene in each parental cultivars were estimated. Then, the analysis was restarted to maximize the likelihood of the other three models (Mendelian, polygenic and environmental model) with the frequency of the major gene fixed. Comparisons of the models were made with likelihood ratio tests. The analysis was performed with pedigree analysis package (PAP) software (Hasstedt 2008) version 6.0. Segregation analysis, implemented in the PAP, has been widely used for estimating the parameters and computing or approximating the likelihood for the mixed model, especially in human genetics (Neuman et al. 1995, Snow and Wijsman 1998, Almasy and Warren 2005).

Results

Acidity in progeny populations was distributed continuously from 0.1 to 1.6 g/100 ml with a peak around 0.5 g/100 ml. Mid-parent values of acidity were very close to family means of that in most of all crosses (Table 1). Acidity in progenies from crosses among cultivars with acidity of 0.3–0.4 g/100 ml was distributed around the mid-parent value, showing a typical normal distribution (Fig. 3a). In crosses in which the acidity of one parent on a cross was in a higher category (from 0.4 to 0.8 g/100 ml), the distribution of acidity shifted towards a higher category (Fig. 3b–c). Progenies with low acidity were also obtained, even though the mid-parent values became higher. These frequency distributions suggested that acidity would be a quantitative trait controlled by polygenes as well as by some major genes with a large effect that made acidity low (< 0.3 g/100 ml) or high (> 0.6 g/100 ml).

The likelihood ratios of the genetic models (mixed, Mendelian and polygenic) to the environmental model were tested,

Table 1: Mid-parent values and family means of acidity in 31 apple families examined

Cross	Mid-parent value (g/100 ml)	Progeny evaluated (no.)	Smallest value in the progeny (g/100 ml)	Largest value in the progeny (g/100 ml)	Family mean (g/100 ml)
Tsugaru × Delicious	0.32	37	0.12	0.49	0.31
Sansa × Delicious	0.34	37	0.13	0.56	0.34
Orin × Sansa	0.34	4	0.16	0.48	0.37
Tsugaru × Sansa	0.35	13	0.24	0.72	0.43
Fuji × Delicious	0.36	11	0.22	0.62	0.36
Kitaro × Tsugaru	0.36	3	0.42	0.58	0.51
Fuji × Orin	0.36	3	0.37	0.54	0.47
Fuji × Kotaro	0.36	11	0.36	0.85	0.53
Fuji × Tsugaru	0.37	4	0.40	0.61	0.51
Senshu × Kotaro	0.37	28	0.17	0.82	0.51
Sansa × Shinsekai	0.38	11	0.32	0.64	0.45
Kitaro × Shinsekai	0.38	7	0.35	0.73	0.55
Toko × Kotaro	0.39	5	0.24	0.83	0.41
Fuji × Sansa	0.39	15	0.19	0.74	0.45
Senshu × Sansa	0.40	16	0.11	0.83	0.46
Senshu × Shinsekai	0.40	13	0.27	0.75	0.50
Fuji × Morioka #54	0.45	14	0.25	0.94	0.55
Senshu × Morioka #54	0.46	13	0.31	0.71	0.47
Fuji × Megumi	0.47	16	0.20	0.73	0.46
Toko × Morioka #54	0.47	5	0.26	0.68	0.39
Akane × Orin	0.47	10	0.15	0.83	0.44
Fuji × Morioka #57	0.49	1	0.78	0.78	0.78
Fuji × Himekami	0.50	1	0.90	0.90	0.90
Sansa × Akane	0.51	22	0.14	1.16	0.51
Senshu × Himekami	0.51	8	0.20	0.86	0.51
Kitaro × Akane	0.51	48	0.27	1.27	0.62
Fuji × Akane	0.52	7	0.24	0.80	0.49
Senshu × Akane	0.53	9	0.17	0.68	0.38
Jonathan × Delicious	0.57	16	0.17	1.06	0.59
Akane × Megumi	0.59	16	0.41	1.17	0.76
Jonathan × Sansa	0.60	4	0.59	1.62	1.06

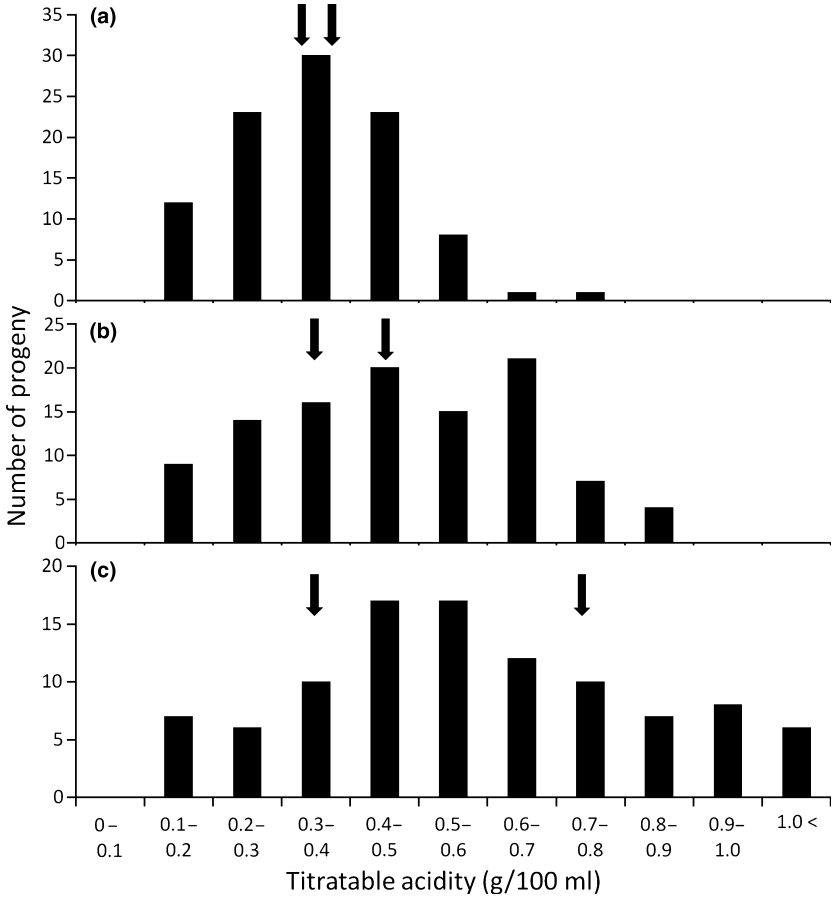

Fig. 3: Frequency distributions of acidity in progeny obtained from crosses (a) between parents with TA at 0.3–0.4 g/100 ml, (b) between parents at 0.3–0.4 g/100 ml and 0.4–0.5 g/100 ml, and (c) between parents at 0.3–0.4 g/100 ml and 0.6–0.8 g/100 ml. The arrows indicate the acidity of the parents. Each group consists of progeny from (a) seven crosses ('Sansa' × 'Delicious', 'Tsugaru' × 'Delicious', 'Sansa' × 'Shinsekai', 'Sansa' × 'Tsugaru', 'Sansa' × 'Orin', 'Kitaro' × 'Shinsekai' and 'Kitaro' × 'Tsugaru'); (b) nine crosses ('Fuji' × 'Delicious', 'Fuji' × 'Kotaro', 'Senshu' × 'Shinsekai', 'Fuji' × 'Sansa', 'Senshu' × 'Kotaro', 'Senshu' × 'Sansa', 'Fuji' × 'Tsugaru' 'Fuji' × 'Orin' and 'Toko' × 'Kotaro'); (c) five crosses ('Sansa' × 'Akane', 'Akane' × 'Orin', 'Kitaro' × 'Akane', 'Jonathan' × 'Delicious' and 'Jonathan' × 'Sansa')

and all tests were highly significant, indicating that the phenotypic variance of acidity was a result of genetic control. Moreover, the likelihood ratio tests for the mixed model vs. Mendelian and polygenic models were also highly significant, indicating that the mixed model was accepted as the model that best fits the observed pedigree data. Therefore, the existence of a major gene was verified in the inheritance of acidity.

When the mixed model was applied to the pedigree data, the genotypic values of the major gene (A^1A^1, A^1A^2 and A^2A^2) were estimated to be 0.45, 0.52 and 0.92 g/100 ml, respectively (Table 2). The values of the homozygote (A^1A^1) and the heterozygote (A^1A^2) were very close and lower than that of the other homozygote (A^2A^2), indicating that an allele (A^1) of the major gene behaved as a completely dominant gene with the function of reducing acidity. Therefore, we designated the A^1 and A^2 alleles as A and a, respectively. Genotypes of the major gene in 15 of 19 parental cultivars were accurately estimated (probability of the estimation was >90%) (Fig. 2). The estimate of heritability after accounting for the major gene was moderately high, 0.43, in the mixed model. This means that even with removal of the effect of the major gene, acidity could fluctuate considerably by the effect of polygenes. The phenotypic values of AA and Aa genotypes in parental cultivars were 0.31–0.56 g/100 ml and 0.37–0.83 g/100 ml, respectively (Fig. 2). Both genotypes had large variation in the phenotypic value, and it is difficult to distinguish the two genotypes only by the phenotypic values. When the effect of the major gene was integrated into the effect of polygenes (Polygenic model), the estimate of heritability became higher, 0.73 (Table 2).

The frequency distributions of progeny were estimated using the parameters obtained (Fig. 4). The progeny of each acid genotype was assumed to be distributed according to a normal distribution defined by the genotypic value and the σ_e, shown in Table 2, as the mean and standard deviation, respectively. A translocation of the distributions from the genotypic mean caused by the effect of polygenes was corrected by the mean phenotypic value of parents, expressed as a deviation from the genotypic mean of the parents, being multiplied by the heritability ($g_p + h^2 \cdot \{(p_f - g_f) + (p_m - g_m)/2\}$; where p_f, phenotypic value of father; p_m, phenotypic value of mother; g_f, genotypic value of father; g_m, genotypic value of mother; g_p, genotypic value of the progeny; h^2, heritability). Frequent distributions estimated from the genotypic and phenotypic values of parents were well fitted to the distributions of progeny actually obtained from the parents. The reason that progenies with both high and low acidity were obtained from

Table 2: Genetic parameters in mixed and polygenic models estimated by the segregation analysis

	Heritability	Genotypic value (g/100 ml)			Standard deviation
Model	h^2	A^1A^1 (AA)	A^1A^2 (Aa)	A^2A^2 (aa)	σ_e
Mixed	0.43	0.45	0.52	0.92	0.201
Polygenic	0.73	—	—	—	0.270

crosses between parents with low and high acidity can be explained by the fact that both parents are heterozygotes (*Aa*) in acidity (Fig. 4d).

The proportion of progeny with adequate acidity, 0.35–0.45 g/100 ml, for dessert use was estimated using the parameters obtained when the mid-parent value was changed in the range of 0.2–0.6 g/100 ml. Because a difference in the genotypic value of acidity was not large between *AA* and *Aa* genotypes, there could be cultivars in which the phenotypic

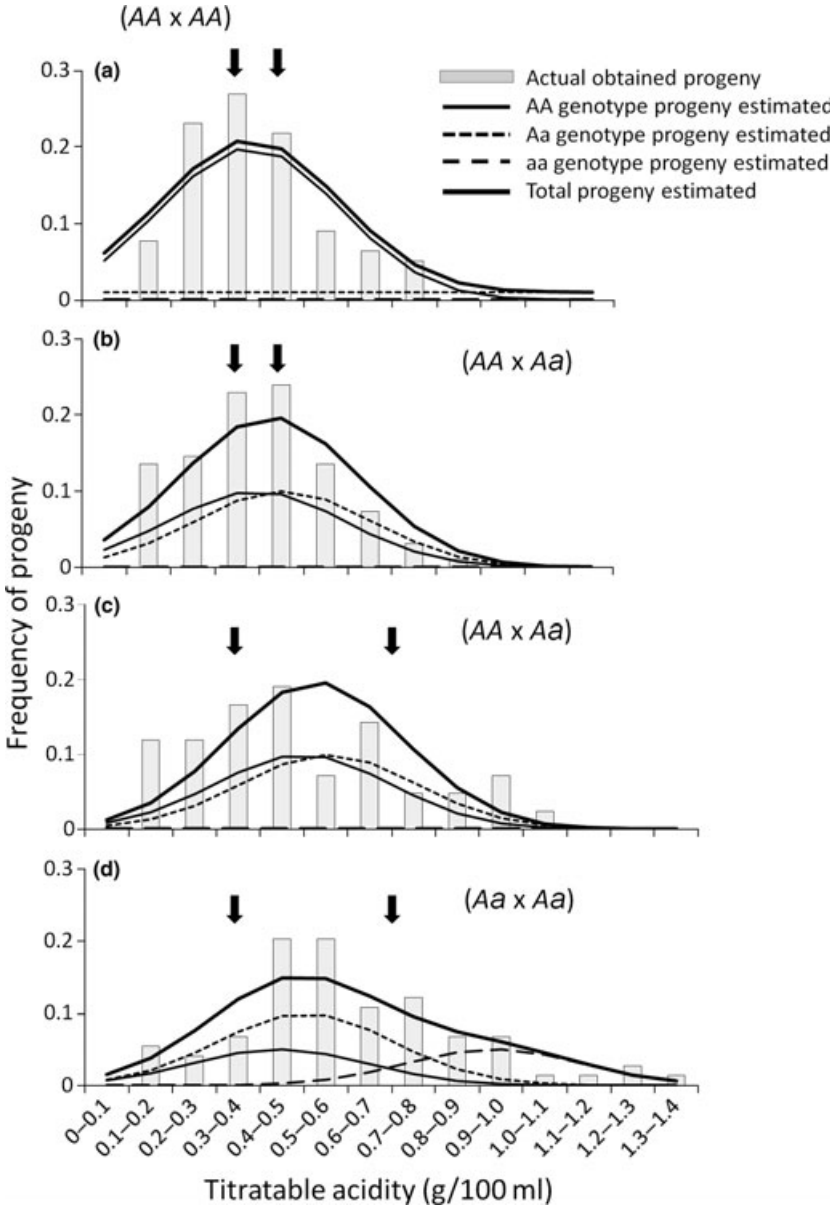

Fig. 4: Comparison of the frequency distributions of acidity in progeny actually obtained with those estimated by genetic parameters reported in Table 2. The progeny was classified into four groups on the basis of the phenotypic and genotypic values of the parents in acidity; (a) from crosses of $AA \times AA$ genotypes with acidity at 0.3–0.5 g/100 ml; (b) from crosses of $AA \times Aa$ genotypes with acidity at 0.3–0.5 g/100 ml; (c) from crosses of $AA \times Aa$ genotypes with acidity at 0.3–0.4 g/100 ml and 0.6–0.8 g/100 ml; (d) from crosses of $Aa \times Aa$ genotypes with acidity at 0.3–0.4 g/100 ml and 0.6–0.8 g/100 ml. The arrows indicate the acidity of the parents. Each group consists of progeny from (a) seven crosses ('Tsugaru' × 'Delicious', 'Kitaro' × 'Shinsekai', 'Kitaro' × 'Tsugaru', 'Fuji' × 'Delicious', 'Senshu' × 'Shinsekai, 'Fuji' × 'Tsugaru' and 'Fuji' × 'Orin'); (b) six crosses ('Sansa' × 'Delicious', 'Orin' × 'Sansa', 'Sansa' × 'Shinsekai', 'Sansa' × 'Tsugaru', 'Senshu' × 'Sansa' and 'Fuji' × 'Sansa'); (c) four crosses ('Akane' × 'Orin', 'Fuji' × 'Akane', 'Senshu' × 'Akane' and 'Jonathan' × 'Delicious'); (d) three crosses ('Sansa' × 'Akane', 'Kitaro' × 'Akane' and 'Jonathan' × 'Sansa')

value was the same but the genotype was different: *AA* and *Aa*. All progenies from crosses *AA* × *AA* or *AA* × *Aa* have a low-acid allele *A*, and the proportion of progeny with 0.35–0.45 g/100 ml in acidity is 19–20% when the mid-parent value is around 0.4 g/100 ml (Fig. 5). On the other hand, the proportion decreased to 14–15% when progenies were obtained from the cross *Aa* × *Aa*. This is because a quarter of the progeny from the cross is the high-acid genotype (*aa*).

Discussion

To the best of our knowledge, a segregation analysis using PAP was not previously conducted in populations of plant breeding. The segregation analysis successfully revealed that fruit acidity in apple was governed by polygenes as well as a major gene. In early studies, a discontinuity was observed in the distribution of acidity in progeny around pH 3.8 or 0.3% in malic acid concentration (Nybom 1959, Visser et al. 1968, Brown and Harvey 1971). The segregation ratio between low-acid (pH > 3.8) and moderate-to-high acid (pH < 3.8) was presumed to be 1 : 3, and the conclusion was that acidity was governed by a major gene, with moderate-to-high acidity being dominant, designated *Ma*. On the other hand, although Visser et al. (1968) observed discontinuous distribution in some progeny, they questioned the segregation ratio 1 : 3 because the segregation ratios varied greatly in the progeny. Afterwards, Visser and Verhaegh (1978) indicated that the mode of inheritance of acidity could be explained by an additive genetic action model (polygene). In our study, no distinct discontinuity in the distribution of acidity was observed in the low-value range, around 0.3 g/100 ml in TA (Fig. 3). One possibility is that parent cultivars used for making crosses in the early studies differ largely from those in our study. Both excessively sweet (< 0.2% in malic acid) and sour (> 0.9%) parent cultivars were used in the early studies (Brown and Harvey 1971). Those are old cultivars that have not been used in modern breeding programmes because of the low quality of the fruit. In our study, TA in parental cultivars ranged from 0.31–0.83 g/100 ml (= %) (Fig. 2). Therefore, acidity in the progeny

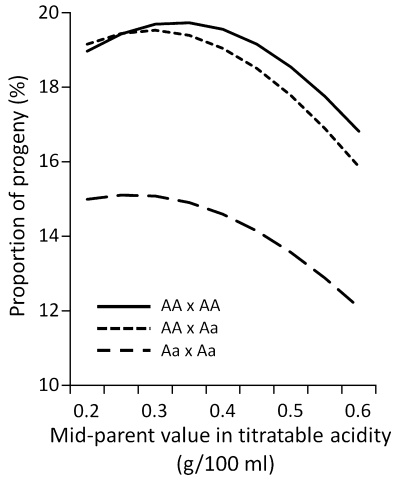

Fig. 5: Proportion of progeny having titratable acidity (TA) at 0.35–0.45 g/100 ml obtained from three combinations of acid genotypes with mid-parent values in the range of 0.2–0.6 g/100 ml TA. The proportion was estimated from the genetic parameters reported in Table 2

obtained from the parents with moderate acidity has a compact range relative to that in the early studies, and excessive low-acid genotypes had probably not been segregated. Kouassi et al. (2009) estimated a heritability of TA at 0.79 using 2207 pedigreed genotypes with 132 founders from breeding programmes in six European countries. In our study, when the effect of a major gene was included in the polygenes, the heritability of TA was 0.73 (Table 2) and nearly identical to the estimate above. One of the reasons is that the constitution of cultivars used in our breeding was similar to that in Europe. All the original parental cultivars in our study are also used in the breeding programmes in Europe, except for 'Indo', which originated in Japan. Noiton and Alspach (1996) analysed the pedigrees of 50 apple cultivars produced from modern breeding programmes around the world and reported that 'Cox's Orange Pippin', 'Golden Delicious', 'Red Delicious', 'Jonathan' and 'McIntosh' were the most frequent ancestors. One of the five cultivars was also an ancestor of each of the progenies used in our study. Therefore, our results are adaptable to modern apple breeding programmes throughout the world.

Recent studies of molecular markers and genetic linkage maps have tried to identify loci closely related to acidity and the positions of the loci on apple linkage maps. Maliepaard et al. (1998) evaluated acidity using pH-indicator paper in a seedling population from 'Prima' × 'Fiesta' under the following assumptions: pH values lower than 3.8 were considered to indicate the presence of the *Ma* gene; and genotypes with pH values > 3.8 were considered to be of the *mama* recessive genotype and the parents, heterozygous *Mama*. The *Ma* gene was then mapped to the distal end of the linkage group (LG) 16. Liebhard et al. (2003) indicated that fruit from trees carrying two acidity alleles on LG8 and LG16 showed an average of 1.12% in TA and fruit from trees carrying no acidity allele showed an average of 0.58% in TA. The values were close to our data that the genotype means of high-acid and moderate-acid types were 0.92 and 0.45–0.52 g/100 ml (%), respectively (Table 2).

Malic acid accumulates in young fruit up to about 1.5 g/100 g fresh weight and decreases during growth and ripening (Ulrich 1970). The difference in malic acid content between a high-acid genotype and a low-acid genotype was unrelated to either malic acid synthesis or the degradation activity of malic enzyme (Beruter 2004). The low content of malic acid in the low-acid genotype was suggested to be the result of a restricted ability to accumulate malic acid in parenchyma cells. Yao et al. (2007) isolated a gene, *Mal-DDNA*, expressed differently between a low- and a high-acid genotype, and the transcription of the *Mal-DDNA* was much higher in genotypes with low acidity than in those with high acidity, indicating that the *Mal-DDNA* was related to the low-acid characteristic. Yao et al. (2009) recently revealed that the activity of malic enzyme, by which malic acid is degraded, was higher in ripening fruit of the low-acid genotype than in that of a high-acid genotype. These results indicate that malic acid is down-regulated by several genetic factors during the ripening stage. A dominant allele of the major gene involved in making acidity lower, estimated in our study, may regulate the ability to accumulate malic acid in parenchyma cells or may promote the degradation of malic acid.

The estimated distributions of acidity were not significantly different between the *AA* and *Aa* genotypes (Fig. 4). However, the proportions of progeny with adequate acidity were significantly different depending on which genotype was used

as parents for the crossing (Fig. 5). In other words, the proportion will be lower when progenies are obtained from a cross of $Aa \times Aa$. Therefore, it is very important to know the genotypes of parental cultivars before crossing. To estimate the genotypes of many parental cultivars, the segregation analysis performed in our study using a large complex pedigree from the breeding population is useful for breeders because only phenotypic records from a pedigreed population are required. The identification of QTL for many characteristics has been thoroughly reported in fruit tree studies. However, molecular markers that are close to the genes underlying the characteristics are only developed using restricted populations derived from crossing between a few cultivars. Therefore, it is uncertain whether the markers can be useful to other cultivars and whether the QTL localized to the maps of a particular cultivar are valid for other cultivars.

In this study, crosses between high-acid genotypes ($Aa \times aa$ or $aa \times aa$) were absent because the objective of our breeding programme was to produce dessert apples. To verify the accuracy of the acid genotypes estimated, it will be necessary to incorporate high-acid cultivars into the pedigree population analysed.

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