

Multivariate mixed inheritance models for QTL detection on porcine chromosome 6

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Summary

A series of multivariate mixed-inheritance models is fitted to the data from an outbred-line pig cross commercially used in Norway. Each model accommodates information on polygenic (co)variances between F_2 individuals and their F_1 parents across the five traits through incorporation of a random animal effect. Considered traits relate to meat quality and are chosen following up the results from a previous evaluation, in which a putative quantitative trait locus (QTL) was identified on chromosome six that affects the amount of intramuscular fat (IMF), meat percentage, meat tenderness and smell intensity (Grindflek *et al.*, 2001). An additional trait included in the model, based on results of other studies, is the backfat thickness. The analysed material comprises data scored for 305 F_2 individuals, whereas marker information is available for F_1 and F_2 generations. Based on the results of the multivariate analysis with the mixed-inheritance model, it was possible to conclude that the evidence for QTLs for meat percentage, meat tenderness and smell intensity in the study of Grindflek *et al.* (2001) do not represent separate QTLs, but is caused by the fact that the applied pre-adjustment of trait values for polygenic effects failed properly to remove the polygenic variation. The QTL effect on IMF on chromosome six was confirmed.

1. Introduction

Recent research results indicate that porcine chromosome six (SSC6) probably hosts genes related to meat quality. Among the most often reported is a quantitative trait locus (QTL) for the amount of intramuscular fat (IMF) (de Koning *et al.*, 1999; Gerbens *et al.*, 1999; de Koning *et al.*, 2000; Gerbens *et al.*, 2000; Óvilo *et al.*, 2000; Grindflek *et al.*, 2001). Additionally, a QTL affecting backfat thickness (BFT) has also been reported within the same chromosomal region (Óvilo *et al.*, 2000; Malek *et al.*, 2001). Results obtained in a Norwegian commercial slaughter pig cross, based on univariate analyses using the Lander & Botstein model (1989), give evidence for a putative QTL for IMF, meat percent, meat tenderness and smell intensity, located on SSC6 (Grindflek *et al.*, 2001).

The usual approach to mapping QTLs for meat quality is to evaluate series of univariate models (i.e. separate analysis for each trait). On one hand, such a strategy is a plausible one, because meat quality in pig is described by a very large number of traits (e.g. 44 traits in the data of Grindflek *et al.*, 2001) and simultaneous analysis of all the traits is practically impossible. From a computational point of view, such a model would have too many dimensions and, statistically, there would be too many parameters to be estimated simultaneously for a limited number of individuals. On the other hand, however, some of the traits are highly correlated, so that QTL detection could profit from increased power and higher accuracy of gene localization by performing a multivariate analysis (Jiang & Zeng, 1995; Henshall & Goddard, 1999; Williams *et al.*, 1999; Knott & Haley, 2000). Moreover, as pointed by Rencher (1998), a univariate model provides only partial information about the genetic architecture underlying meat quality, because it ignores other traits that are also related to

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the phenomenon under study. Finally, performing several separate univariate analyses increases the probability of type I error, which is usually not realized and consequently not accounted for (Caliński *et al.*, 2000). Mixed models for QTL detection in line-cross data have been proposed by Rathje *et al.* (1997), Pérez-Enciso & Varona (2000), Szyda *et al.* (2000, 2002) and Nagamine & Haley (2001). Multivariate methods have been applied as an extension to regression interval mapping (Haley & Knott, 1992) by Knott & Haley (2000) and Hackett *et al.* (2001), to composite interval mapping of Zeng (1994) by Jiang & Zeng (1995) and Caliński *et al.* (2000), and in the form of principal-component analysis by Weller *et al.* (1996) and Mangin *et al.* (1998).

The objective of the current study was to refine the results of Grindflek *et al.* (2001) by applying a series of multivariate mixed-inheritance models covering all the five traits of main interest on SSC6 (i.e. IMF, meat percentage, meat tenderness, smell intensity and BFT). One of the most important gains from multivariate modelling, apart from enhanced estimation accuracy and detection power, is the possibility of distinguishing between linked and pleiotropic QTL scenarios. Additional power and accuracy are provided by the differentiation between polygenic and QTL-based components of genetic variance directly in the model (as shown by, for example, Nagamine & Haley, 2001). Consequently, no precorrection of parental effects is needed, which might often be inaccurate, especially if there are few parents in the pedigree.

2. Materials

(i) Phenotypic information

Animals in this experiment originate from a cross commercially used for slaughter pigs in Norway. The cross was designed by mating five Duroc boars to five Norwegian Landrace sows in the parental generation to produce five Landrace–Duroc boars (F_1). Each of the F_1 boars was then mated to five Norwegian Slaughter Pig cross sows (50% Norwegian Landrace, 50% Yorkshire) to produce 305 F_2 individuals that were recorded for a number of meat quality and carcass traits. In the current study, of 44 traits describing meat quality, five quantitative measurements (IMF, meat percent, meat tenderness, smell intensity and BFT) are considered. IMF was determined from samples from the longissimus dorsi muscle by the Foss–Let method (Woodward *et al.*, 1976), meat percentage was measured by GP2 analyses and BFT measurements were taken between fourth and fifth ribs. The meat tenderness (scored by chewing force) and smell intensity were evaluated by a trained taste panel of ten assessors, who scored the traits on a scale from 1 (no intensity) to 9 (distinct intensity). Details

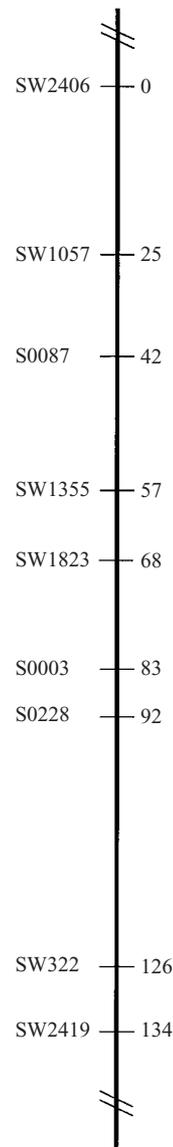


Fig. 1. Genetic linkage map for SSC6 from the study of Grindflek *et al.* (2001).

about the cross and trait recording are given by Grindflek *et al.* (2001).

(ii) Genotypic information

Marker genotypes are available for F_1 and F_2 individuals. The marker map on SSC6 consists of nine markers and has already been described (Grindflek *et al.*, 2001). Marker linkage analysis was performed using the CRIMAP package version 2.4 (Green *et al.*, 1990). The CHROMPIC option was used to search for unlikely double crossovers, and the map for SSC6 (Fig. 1) was constructed with the BUILD option of the program.

The assumption underlying the configuration of genotypes in the available data set is that both paternal lines in a cross are fully homozygous both for markers

Table 1. Polygenic and residual variances and correlations between meat quality traits. Numbers in roman text show polygenic effects, whereas those in italics show residual effects. The numbers on the diagonal are polygenic and residual variances. Numbers above the diagonal are polygenic correlations and those below are residual correlations between considered meat-quality traits, estimated based on the full model

	IMF	Meat percentage	Meat tenderness	Smell intensity	BFT
IMF	-0.22 <i>-0.22</i>	-0.45	-0.97	-0.13	-0.43
Meat percentage	<i>-0.01</i>	-3.44 <i>-9.11</i>	-0.33	-0.45	-0.22
Meat tenderness	<i>-0.18</i>	<i>-0.07</i>	-10.54 <i>-21.43</i>	-0.39	-0.47
Smell intensity	<i>-0.07</i>	<i>-0.22</i>	<i>-0.25</i>	-1.96 <i>-0.99</i>	-0.32
BFT	<i>-0.01</i>	<i>-0.06</i>	<i>-0.03</i>	<i>-0.65</i>	-7.62 <i>-2.04</i>

and for a QTL. As a consequence, F₁ sires are expected to be fully informative (i.e. heterozygous) for all the loci considered. However, this is an ideal situation and so, for the practical analysis of our data, the following approximations are set:

- (a) possible multiple alleles of a putative QTL are divided into two categories – favourable (Q) and unfavourable (q), so that the practical analysis relies on a biallelic QTL
- (b) all dams mated to F₁ sires are homozygous at a putative QTL (qq)
- (c) based on marker information from dams, offspring and sires, the marker haplotype phase of F₁ sires is known without error
- (d) for a given F₁ sire, a favourable QTL allele is assigned to the marker haplotype associated with a higher phenotypic mean value of offspring with this haplotype; the other haplotype is assigned an unfavourable QTL allele.

Corresponding probabilities of a given QTL genotype in F₂ animals, which are equivalent to paternal QTL allele transmission probabilities, are given by

$$P(Qq | M_i, Qq_{Si}, qq_{Di}, r) = P(Q | M_i, Qq_{Si}, qq_{Di}, r) = P(Q | M_i, r)P(Qq_{Si})P(qq_{Di})$$

$$P(qq | M_i, Qq_{Si}, qq_{Di}, r) = P(q | M_i, Qq_{Si}, qq_{Di}, r) = P(q | M_i, r)P(Qq_{Si})P(qq_{Di})$$

where M_i is the set of marker information for F₂ individual i comprising marker genotype of a sire, a dam and its own genotype, Qq_{Si} and qq_{Di} are the assumed genotypes at a putative QTL of a sire and a dam (respectively) of individual i , and r is a set of recombination rates between both markers or between a marker and a putative QTL. In the current analysis, $P(Qq_{Si}) = P(qq_{Di}) = 1$, it is however, possible to relax

the assumption that a given sire is heterozygous at a putative QTL by modelling $P(Qq_{Si})$.

(iii) *Statistical model*

QTL mapping was based on the mixed inheritance model (Szyda *et al.*, 2000): $y = X\beta + X_qq + Z\alpha + e$. Here it is assumed that y follows a multivariate normal distribution: $y \sim MVN_p(X\beta + X_qq + Z\alpha + e)$, where p is the number of traits considered, which is five (IMF, meat percentage, meat tenderness, smell intensity and BFT) for the current study. The model components can be partitioned as follows:

$y = [y_1 \dots y_p]^T$, where y is the vector of phenotypic values for p traits considered,
 $\beta = [\beta_1 \dots \beta_p]^T$, where β is the vector of fixed effects other than QTL for p traits, with $\beta_i = [\mu \text{ sex}]^T$,
 $q = [q_1 \dots q_p]^T$, where q is the vector of fixed QTL effects on p traits expressed as a difference between a heterozygous (Qq) and a homozygous (qq) genotype,
 $\alpha = [\alpha_1 \dots \alpha_p]^T$, where α is the vector of random additive genetic effect of each of n F₁ and F₂ individuals for p traits, with $\alpha = [\alpha_1 \dots \alpha_n]^T$,
 e is the vector of random errors,
 X, X_q and Z represent appropriate design matrices,

$$G = \begin{bmatrix} G_{11} & \dots & G_{1p} \\ \vdots & \ddots & \vdots \\ G_{p1} & \dots & G_{pp} \end{bmatrix},$$

where G_{ij} is the polygenic (co)variance matrix for traits i and j ,

$$R = \begin{bmatrix} R_{11} & \dots & R_{1p} \\ \vdots & \ddots & \vdots \\ R_{p1} & \dots & R_{pp} \end{bmatrix},$$

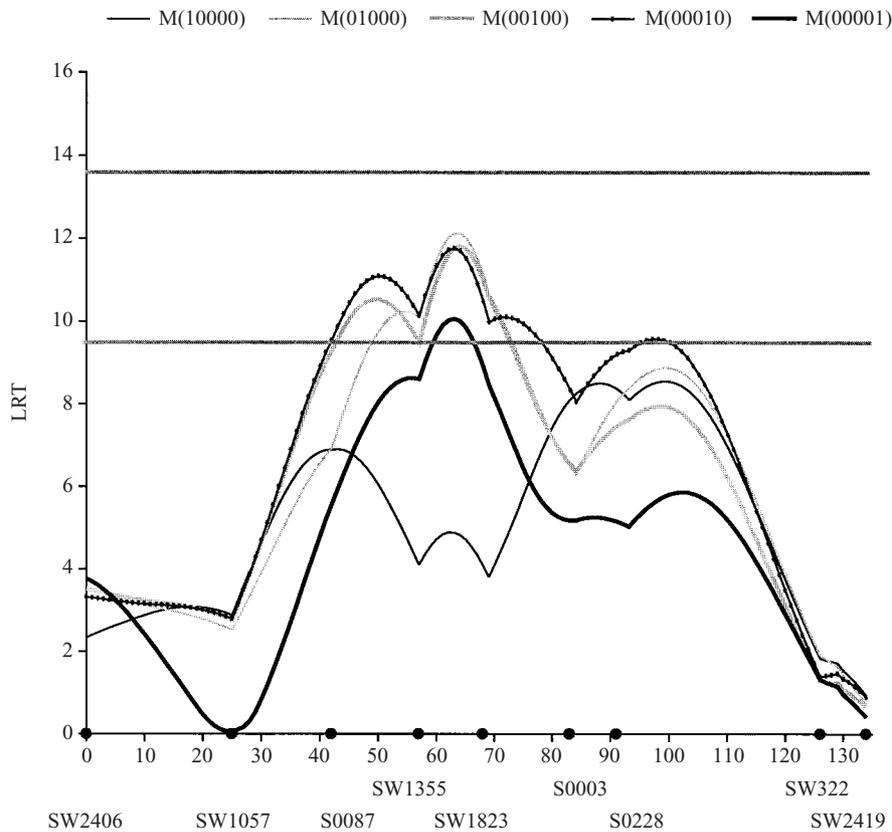


Fig. 2. Likelihood-ratio test profiles for the comparison of M(11111) with each of five single-trait QTL models. The horizontal lines correspond to the 0.05 probability of type I error, with the solid line referring to a chromosomewise level corrected for the effective number of marker intervals and the dashed line to an uncorrected, nominal level. Dots represent relative marker positions. M(10000) is concerned with IMF; M(01000) is concerned with meat percentage; M(00100) is concerned with meat tenderness; M(00010) is concerned with smell intensity; M(00001) is concerned with BFT.

where \mathbf{R}_{ij} is the residual (co)variance matrix for traits i and j .

QTL genotype probabilities in \mathbf{X}_q are calculated following the multiple-marker approach of Knott *et al.* (1996) every 1 cM along the marked chromosome region.

Additionally, a bivariate two-QTL model ($\mathbf{y} = \mathbf{X}\beta + \mathbf{X}_q^{IMF} \mathbf{q}^{IMF} + \mathbf{X}_q^{BFT} \mathbf{q}^{BFT} + \mathbf{Z}\alpha + \mathbf{e}$) was applied to construct a likelihood surface for data on IMF and BFT. Here, \mathbf{q}^{IMF} and \mathbf{q}^{BFT} represent the QTL effect on IMF and BFT, respectively, with corresponding design matrices \mathbf{X}_q^{IMF} and \mathbf{X}_q^{BFT} .

(iv) Hypothesis testing

As a testing criterion the likelihood ratio test statistic is used

$$\lambda = -2 \ln \frac{L(M_0)}{L(M_1)} \sim \chi^2$$

where, $L(M_0)$ and $L(M_1)$ are the maximum values of a likelihood function underlying a more-parsimonious model corresponding to the null hypothesis (H_0) and an unrestricted model, respectively. Model parsimony

is defined in terms of the number of traits affected by a fitted QTL, whereas the other parameters (i.e. β and α) are the same for all the models. Consequently, the full model labelled as M(11111) assumes that $\mathbf{q} = [\mathbf{q}_1 \ \mathbf{q}_2 \ \mathbf{q}_3 \ \mathbf{q}_4 \ \mathbf{q}_5]^T$, where the sequence of traits considered in \mathbf{q} is IMF, meat percentage, meat tenderness, smell intensity and BFT. Testing procedure follows Knott & Haley (2000) extended from two to five dimensional models, considering the following configurations of null hypotheses.

- (1) Comparing the unrestricted model to models assuming a QTL affecting a single trait

$H_0: \mathbf{q} = [\mathbf{q}_1 \ 0 \ 0 \ 0 \ 0]^T$, with the corresponding model labelled as M(10000)

$H_0: \mathbf{q} = [0 \ \mathbf{q}_2 \ 0 \ 0 \ 0]^T$, with the corresponding model labelled as M(01000)

$H_0: \mathbf{q} = [0 \ 0 \ \mathbf{q}_3 \ 0 \ 0]^T$, with the corresponding model labelled as M(00100)

$H_0: \mathbf{q} = [0 \ 0 \ 0 \ \mathbf{q}_4 \ 0]^T$, with the corresponding model labelled as M(00010)

$H_0: \mathbf{q} = [0 \ 0 \ 0 \ 0 \ \mathbf{q}_5]^T$, with the corresponding model labelled as M(00001)

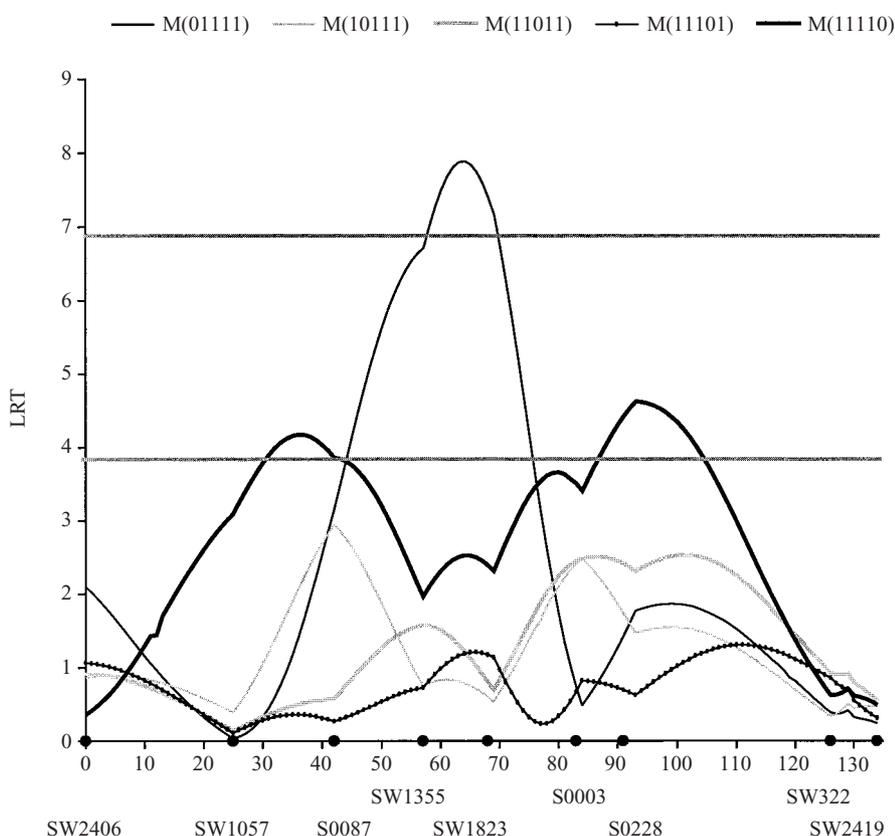


Fig. 3. Likelihood-ratio test profiles for the comparison of M(11111) with each of five four-trait QTL models. The horizontal lines correspond to the 0.05 probability of type I error, with the solid line referring to a chromosomewise level corrected for the effective number of marker intervals and the dashed line to an uncorrected, nominal level. Dots represent relative marker positions. M(01111) is concerned with IMF; M(10111) is concerned with meat percentage; M(11011) is concerned with meat tenderness; M(11101) is concerned with smell intensity; M(11110) is concerned with BFT.

(2) Comparing the unrestricted model to models assuming a QTL affecting four traits

- $H_0: \mathbf{q} = [0 \ \mathbf{q}_2 \ \mathbf{q}_3 \ \mathbf{q}_4 \ \mathbf{q}_5]^T$, with the corresponding model labelled as M(01111)
- $H_0: \mathbf{q} = [\mathbf{q}_1 \ 0 \ \mathbf{q}_3 \ \mathbf{q}_4 \ \mathbf{q}_5]^T$, with the corresponding model labelled as M(10111)
- $H_0: \mathbf{q} = [\mathbf{q}_1 \ \mathbf{q}_2 \ 0 \ \mathbf{q}_4 \ \mathbf{q}_5]^T$, with the corresponding model labelled as M(11011)
- $H_0: \mathbf{q} = [\mathbf{q}_1 \ \mathbf{q}_2 \ \mathbf{q}_3 \ 0 \ \mathbf{q}_5]^T$, with the corresponding model labelled as M(11101)
- $H_0: \mathbf{q} = [\mathbf{q}_1 \ \mathbf{q}_2 \ \mathbf{q}_3 \ \mathbf{q}_4 \ 0]^T$, with the corresponding model labelled as M(11110)

Following Cheverud (2001), the Bonferroni correction was used to approximate the chromosomewise significance level (α):

$$\alpha^* = 1 - (1 - \alpha)^{1/M^*}$$

where α^* represents the nominal type I error rate and M^* is the effective number of marker intervals tested. Practically, M^* corresponds to the actual number of intervals corrected for their intercorrelation, which is expressed by the variance of eigenvalues of the interval

correlation matrix. Unlike Cheverud (2001) but following Jiang & Zeng (1995), a marker interval, not a marker itself, was considered here as an independent testing unit.

For the bivariate two-QTL model, the null hypothesis of interest is that both traits are affected by the same QTL and is given by $H_0: \theta_{IMF} = \theta_{BFT}$, where θ_{IMF} and θ_{BFT} represent positions of QTLs affecting IMF and BFT, respectively, expressed in cM from the leftmost marker. It was tested by assessing a joint confidence interval (CI) for both parameters. A joint rectangular CI results from the intersection of CIs corresponding to both QTL positions obtained using a standard method

$$\hat{\theta} \pm z_{\alpha/2} \sigma_{\hat{\theta}}$$

where $\hat{\theta}$ represents the parameter in question, $z_{\alpha/2}$ is the standard normal deviate corresponding to $\alpha/2$ type I error, and $\sigma_{\hat{\theta}}$ is the standard deviation of $\hat{\theta}$, estimated based on the curvature of the likelihood surface around its maximum following Meyer & Hill (1992; see for example Szyda *et al.*, 2002). Although straightforward to calculate, it ignores the covariance between θ_{IMF} and θ_{BFT} . The exact elliptical CI that would

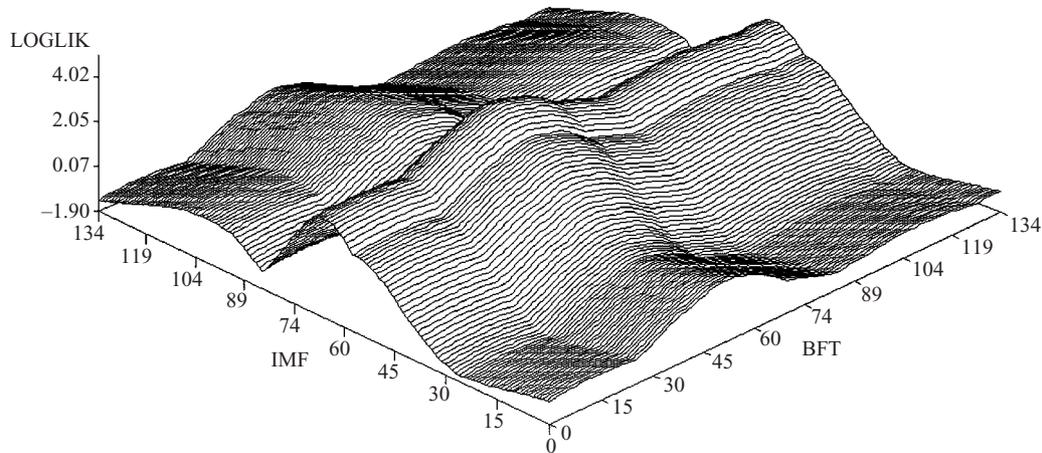


Fig. 4. Likelihood surface for the bivariate two-QTL model for IMF and BFT.

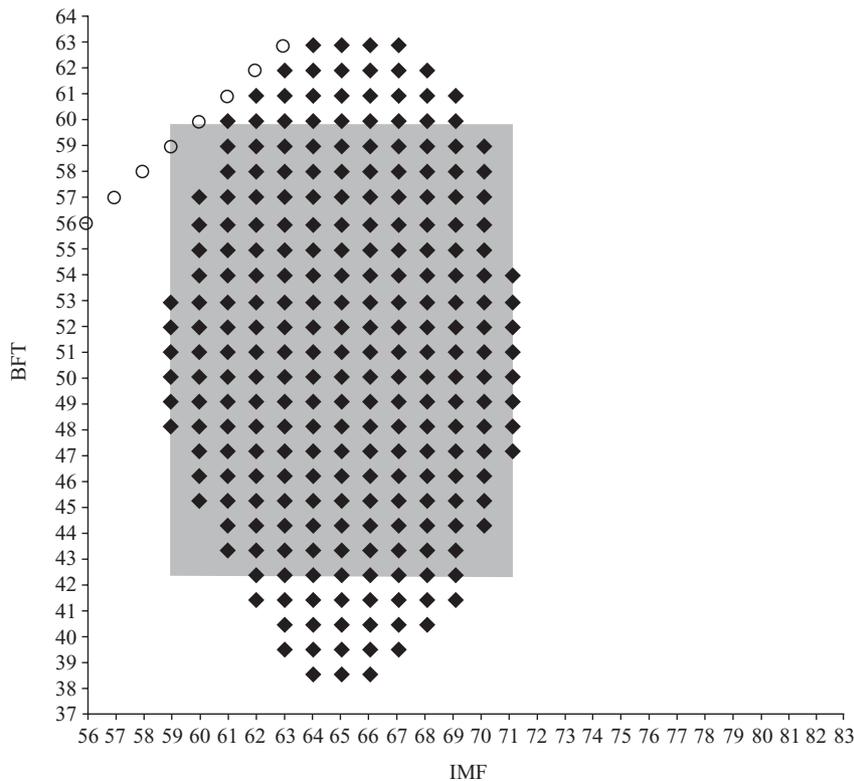


Fig. 5. Rectangular and elliptical 95% confidence intervals for QTL position based on the two-dimensional likelihood surface. Circles mark the diagonal line corresponding to the identical estimates of QTL position for IMF and BFT.

account for the covariance is difficult to determine algebraically. The information about quality of approximation of an exact, elliptical CI through the rectangular CI is contained in the parameter (co) variance matrix, so that the square root of its determinant amounts to the area of the rectangular CI, which corresponds to the elliptical CI (Draper & Smith, 1998). If both areas are similar, the elliptical CI can be well represented by the two-dimensional surface of the likelihood level corresponding to the area covered by the rectangle.

(v) Estimation

The restricted maximum likelihood (REML) approach is applied for the simultaneous estimation of model parameters denoting both, effects and variance components. The corresponding log-likelihood function ($\ln L$) is given by

$$\begin{aligned} \ln L = & -0.5(n-r) \ln(2\pi) \\ & -0.5(\ln|\mathbf{R}| + \ln|\mathbf{G}| - \ln|\mathbf{C}| \\ & + \mathbf{y}^T \mathbf{R}^{-1} \mathbf{y} - \mathbf{y}^T \mathbf{R}^{-1} \mathbf{X}^* \mathbf{b} - \mathbf{y}^T \mathbf{R}^{-1} \mathbf{Z} \mathbf{a}), \end{aligned}$$

where n is the number of records, r is the rank of \mathbf{X}^* (the design matrix for \mathbf{b}) and \mathbf{C} is the coefficient matrix of the mixed-model equations. All the model parameters were estimated at each putative QTL location, building a likelihood profile along the chromosome. The evaluation of REML and parameter estimation involves implementation of the PEST/VCE package (Neumaier & Groeneveld, 1998). Values of REML provided by the software are not directly comparable between different models, which poses a problem for hypotheses testing. In PEST/VCE, computed REML values lack the first term of a full REML and are shifted by a constant depending on the dimension of a model. This procedure alters the level (but not the shape) of the likelihood surface between models differing in parameterization. Although the first likelihood term can be evaluated algebraically, the exact numerical evaluation of the constant is not possible (A. Neumaier, personal communication). Consequently, for the purpose of our study an empirical approach was adopted, in which each model's REML was evaluated for 100 data sets simulated with the size and structure identical to the real data set, but assuming no QTLs. The average REML out of such 100 evaluations is an estimate of the constant.

3. Results

(i) Correlation estimates

Polygenic and residual variances and correlations between considered traits estimated under the full model and corresponding to the highest REML value along the chromosome are given in Table 1. The polygenic correlations vary between 0.97 for meat tenderness and IMF, and 0.13 for IMF and smell intensity. The corresponding residuals are less correlated, with the weakest correlation of -0.01 for IMF and meat percentage, and for IMF and BFT, and the strongest correlation of -0.65 for smell intensity and BFT. Owing to the relatively small sample size, the estimates are subject to large standard errors, but the sign of polygenic correlations reveals trait physiological background.

(ii) QTL mapping

The comparison of the unrestricted and single-trait QTL models is shown in Fig. 2. The likelihood-ratio test profiles corresponding to models M(01000), M(00100), M(00010) and M(00001) all show very similar shapes along the chromosome. The highest prevalence of the full model over the four above models is observed for marker interval SW1355–SW1823, for which the test statistic exceeds the nominal 0.05 probability of type I error (although it does

not reach the chromosomewise threshold). To the contrary, the likelihood-ratio test profile related to the comparison of the full model to M(10000) has a distinct shape that shows no significant differences in fit between the compared models.

The likelihood ratio test profiles summarizing differences in fit between the full model M(11111) and each of the four-trait QTL models M(01111), M(10111), M(11011), M(11101) and M(11110) are given in Fig. 3. Again, the likelihood profile corresponding to M(01111) shows a distinct shape with chromosomewise significant prevalence of M(11111) observed within the region marked by SW1355–SW1823. Considering the nominal type I error rate, M(11110) also appears to be significantly worse than the full model within the two intervals SW1057–S0087 and S0228–SW322. The other models appear not to fit significantly worse. The effective number of marker intervals used to obtain the chromosomewise significance level is 5.85.

From a commercial pig-breeding point of view, it is especially important to determine whether a QTL that significantly affects IMF also has a significant effect on BFT. This was done through a bivariate two-QTL analysis. The resulting likelihood surface is presented in Fig. 4. The rectangular and elliptical 95% CIs for the QTL position corresponding to the surface (Fig. 5) show 0.99991 correspondence, which mainly results from low correlation of 0.014 between estimates of position along the IMF and the BFT axes. The elliptical CI does not contain any of the diagonal points indicating the same positions for QTL for IMF and BFT.

4. Discussion

The original results of QTL detection for meat-quality traits for this data set, based on univariate models, reported four putative QTLs or a single QTL affecting four traits mapped to SSC6 (Grindflek *et al.*, 2001). Furthermore, Grindflek *et al.* (2001) found no evidence of QTLs for BFT, which is a valuable result for breeding practice but contradicts some previous findings, in which QTLs for both, IMF and BFT were reported in this region of SSC6 (Óvilo *et al.*, 2000).

Applying multivariate modelling to the data enabled us to exclude previous results for IMF, meat tenderness, meat percentage and smell intensity as being affected by a single pleiotropic QTL. This conclusion can be drawn from a comparison of models M(11111) and M(10000). As shown in Fig. 2, M(10000) assumes that a QTL affecting IMF does not have a significantly worse fit than the five-trait pleiotropic QTL model, meaning that it is sufficient for statistical description of the data. However, none of the other single-trait QTL models fitted is adequate. Furthermore, as shown in Fig. 3, even if QTL effects

are fitted for meat tenderness, meat percentage, smell intensity and BFT, such a model does not reflect the observed phenotypic variation as long as it lacks a QTL effect on IMF. Based on the significance pattern of likelihood profiles, it is also evident that no separate, linked QTLs for meat tenderness, meat percentage, smell intensity and BFT are expected in the region flanked by SW1355 and SW1823. In the current study, an additional analysis was made to dissect the genetic background between IMF and BFT. Both the shape of the likelihood surface (which shows enhanced likelihood along the whole length of the axis corresponding to BFT) and the elliptical area of 95% CI for QTL position (which does not contain the diagonal with equal position estimates for BFT and IMF) indicate that the QTL affecting IMF has no effect on BFT. Summarizing, three out of four QTLs indicated by the univariate analyses appear to be false positives. Results based on a multivariate model show that the QTL located on SSC6 in the region marked by SW1355 and SW1823 only affects IMF. The observed discrepancy between the univariate fixed-effects model of Grindflek *et al.* (2001) and multivariate mixed-inheritance model QTL mapping shows that precorrection of parental effects applied by Grindflek *et al.* (2001) to the data before using univariate models fails to remove the polygenic background of the trait variation properly. Reanalyses of these data by the univariate approach without precorrection of the data confirms that a QTL effect is only apparent for IMF (results not shown).

A single-trait mixed-inheritance model very similar to the model of Szyda *et al.* (2000), which we now extend to the multivariate case, was recently presented by Nagamine & Haley (2001). Although the two models are almost identical in parameterization, they differ considerably in estimation and hypothesis testing. The estimation procedure used by Nagamine & Haley (2001) is based on the assumption that the polygenic (co)variance is independent of the QTL position tested and thus uses the same estimates throughout the whole chromosome. The convergence of these estimates is then achieved by iterations over the whole chromosome. Here, a standard approach is used in which all the parameters are estimated simultaneously at each putative QTL location along the chromosome. Pérez-Enciso & Varona (2000) take an intermediate way by modelling (co)variances conditionally on the available marker information (i.e. separately for each chromosome segment). Hypothesis testing is based on the F statistics in Nagamine & Haley (2001) and on λ (the likelihood-ratio test) here. The advantage of using the F test is that the empirical evaluation of the numerical constant of REML is not needed. However, a test involving only one of model parameters (σ_e^2) is likely to provide biased results when σ_e^2 is biased. Thus, using λ involving values of REML based on all the model parameters seems to be more

robust approach to inaccurate or biased estimation. More research is needed to justify the above considerations.

It is important to stress that our model is a pleiotropic QTL model, and so inferences concerning the presence of linked QTLs are not based on formal testing and can only be restricted to the regions of clear significance pattern of differences in fit between alternative models. Based on a stepwise selection procedure, Zeng (1994) used additional markers as cofactors and Hackett *et al.* (2001) fitted an infinitesimal model of all possible QTL locations. Furthermore, Jiang & Zeng (1995) followed by Caliński *et al.* (2000) fitted two-QTL models by introducing recombination parameter between flanking markers and two (instead of one) putative linked QTL positions. These approaches are capable of identifying linked QTLs within multivariate framework but they lack the polygenic component of genetic variation. Also the analysis of correlated traits based on principal components applied to interval mapping by Mangin *et al.* (1998) provides much flexibility in terms of hypotheses testing. However, as pointed by Hackett *et al.* (2001), inferences based on principal components might result in the detection of spurious QTLs.

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