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Abstract

Inherited traits are often influenced by several genes. Regions on the genome known to affect such traits are called Quantitative Trait Loci (QTL). In variance component QTL analysis, a mixed model is used to detect the most likely chromosome position of a QTL. The putative QTL is included as a random effect and a method is needed to estimate the QTL variance. The standard estimation method used is an iterative method based on the Restricted Maximum Likelihood (REML). In this paper, we present a novel non-iterative variance component estimation method. This method is based on Henderson's (1953) method 3, but relaxes the condition of unbiasedness. Two similar estimators were compared, which were developed from two different partitions of the sum of squares in Henderson's method 3. The approach was compared to REML on data from a European wild boar $\times$ domestic pig intercross. A meat quality trait was studied on chromosome 6 where a functional gene was known to be located. Both partitions resulted in estimated QTL variances close to the REML estimates. From the non-iterative estimates we could also compute good approximations of the likelihood ratio curve on the studied chromosome.

Keywords: Variance components, REML, Henderson's method 3, minimized mean square error, QTL analysis.

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1 Introduction

Regions on the genome known to affect continuous traits are called Quantitative Trait Loci (QTL). In animal experimental data, breeds that are expected to differ genetically are crossed. These data are commonly analyzed using a simple regression model that assumes no genetic variation between individuals of the same breed (Haley and Knott 1992, Broman 1997). Animal breeds are known to vary genetically, and the within breed variation may be modeled as a random effect (Perez-Enciso and Varona 2000, Rönnegård and Carlborg 2006). The variance component estimation can be extremely computationally demanding because the model is fitted at every tested location (often > 1000) on the genome.

In a variance component QTL analysis all the founders of the analyzed families are assumed unrelated with genes randomly sampled from an outbred population. Here the QTL effects are modeled as a random effect in a mixed linear model (Goldgar 1990, Blangero et al. 2001). The variance components of this model have so far been estimated using iterative maximum likelihood based algorithms. The two most commonly used methods are maximum likelihood estimation with Fisher's scoring (see e.g., Pawitan 2001) and restricted maximum likelihood estimation with average information REML (Johnson and Thompson 1995) that combines Newton method and Fisher's scoring. The power to detect QTL is considerably higher in controlled animal crosses than in e.g., human data. The computational demands are lower in human data, where many small and independent families are analyzed, than in animal crosses, where most animals are related in a single pedigree. Numerical methods to speed up the variance component estimation using REML in animal crosses with a small number of founders have recently been developed (Rönnegård et al. 2007), but the REML estimation is still very computationally demanding and depends on good initial values in the iterative procedure to converge within a limited number of iterations.

Henderson (1953) developed non-iterative methods that gives unbiased variance component estimators. In our paper, we will concentrate on Henderson's method 3, which allows for fixed, random and interaction effects in the model. A problem with this method is that the estimates can assume negative values and the properties of the estimators are inferior to REML. For a balanced linear mixed model Kelly and Mathew (1993) improved the unbiased ANOVA estimator such that the resulting estimator had smaller MSE and smaller probability of negativity than the ANOVA estimator. Kelly and Mathew (1994) presented several nonnegative estimators for mixed models with unbalanced data. The models they considered consisted of two variance

components, where one of the components is the error variance and the other variance component is the parameter of interest. If additional variance components were to be included in their model then these were treated as nuisance parameters and were deleted from the model by orthogonal projections.

Following the ideas of Kelly and Mathew (1993,1994), for mixed models with three variance components Henderson's method 3 has been improved by Al-Sarraj and von Rosen (2007). The variance component estimator corresponding to the QTL was modified such that the leading terms of the MSE were minimized.

The aim of this study is to test the utility of modified Henderson's 3 estimates in a QTL study. Two modified estimators based on Henderson's method 3 are compared; all the variance components are included in the first estimator, whereas the second estimator only includes two variance components, i.e., the model is reduced by a suitable linear transformation (following Kelly and Mathew 1993). The methods are tested on data from an experimental cross between wild and domestic pigs. The estimates are also compared with REML estimates obtained from the same data.

2 QTL variance component model

The aim of a QTL analysis is to detect regions most likely to harbor genes affecting the studied trait. In the data set that we analyze the functional halothane gene has previously been identified, which means that we know the position and can compare estimated position from our QTL analysis with the true position.

We use a variance component model in our QTL analysis. Let Y be the $n \times 1$ trait vector that may also be influenced by fixed effects such as sex, age, etc. Moreover, the correlation between trait values is often affected by common family environments. Hence, this can be represented by the following mixed linear model

$$Y = X\beta + Z_1 u_1 + Z_2 u_2 + e, \tag{1}$$

where Y is multivariate normal, β is a $c \times 1$ vector of fixed effects and X is a known $n \times c$ design matrix. The first random effect of (1), u_1, is $m \times 1$ vector of independently normally distributed base generation allele effects, i.e., $u_1 \sim MVN(0, \frac{1}{2}\sigma_1^2 I)$ where I is the identity matrix and σ_1^2 is the QTL genotypic variance. The genotypic value v_i of individual i in the base generation is the sum of the pair of QTL allele effects at a specific position $v_i = u_k + u_{k+1}$, where the QTL alleles are arbitrarily numbered $k = 2i - 1$ in the base. Hence,

by defining the variance of the random QTL genotypic effects as σ_1^2, the variance of the QTL allele effects is $\frac{1}{2}\sigma_1^2$. The QTL alleles are all assumed to be independent in the base generation, i.e., $\mathrm{Cov}(u_i, u_j) = 0$ where i and j are different indices for the m base alleles. The number of base generation alleles m equals twice the number of base generation individuals. Z_1 is the $n \times m$ incidence matrix giving the two base generation alleles that have been inherited by a specific individual. Furthermore, the second random effect represented in (1) by u_2 is the $q \times 1$ vector of family effects, $u_2 \sim \mathrm{MVN}(0, I\sigma_2^2)$ and Z_2 is the corresponding $n \times q$ incidence matrix. e is the $n \times 1$ vector of random error with $e \sim \mathrm{MVN}(0, I\sigma_e^2)$ where σ_e^2 is the error variance. The variance-covariance matrix of Y is therefore:

$$V = \frac{1}{2} Z_1 Z_1' \sigma_1^2 + Z_2 Z_2' \sigma_2^2 + I\sigma_e^2 \tag{2}$$

where $0.5 Z_1 Z_1'$ is the identity-by-descent (IBD) matrix Π. The flow of alleles through the pedigree is generally not ambiguously known and has to be calculated from genetic marker information. Instructions and algorithms for calculating Π are found in Almasy and Blangero (1998), Fernando and Grossman (1989), Goldgar (1990).

In our study we used a deterministic method (Pong-Wong et al. 2001) to calculate the IBD matrix at every 5 cM along pig chromosome 6. Z_1 was then calculated from single-value decomposition of Π.

3 Modified Henderson's method 3

In Henderson's method 3, the mean squares associated with various ANOVA tables are set equal to their expectation, and estimates are obtained by solving the resulting linear equations. The set of equations are not uniquely defined since there are more reduction sums of squares than variance components. We will study two cases which we will refer to as Partition I and Partition II. In Partition I, all three variance components are included, whereas only σ_1^2 and σ_e^2 are included in Partition II. The latter partitioning is similar to the case studied by Kelly and Mathew (1993). The obtained variance component estimators from the two partitions are given in Appendix A. Modified estimators are then obtained by perturbing the standard estimator, such that the obtained estimator has a MSE that is less than the unmodified one (for details see Al-Sarraj and von Rosen 2007).

The modified estimator from Partition I is given by:

$$\begin{aligned}\widehat{\sigma}_1^2 &= c_1\Big(\frac{Y'(P_{x1}-P_x)Y}{tr(P_{x1}-P_x)V_1} - \frac{tr(P_{x1}-P_x)V_2 d_1 Y'(P_{x_{12}}-P_{x1})Y}{tr(P_{x1}-P_x)V_1 tr(P_{x_{12}}-P_{x1})V_2} \\ &\quad + \frac{k d_2 Y'(I-P_{x_{12}})Y}{tr(P_{x_{12}-P_{x1}})V_2 tr(I-P_{x_{12}})}\Big). \qquad (3)\end{aligned}$$

where $k = tr((P_{x1}-P_x)V_2)tr(P_{x_{12}}-P_{x1}) - tr(P_{x1}-P_x)tr((P_{x_{12}}-P_{x1})V_2)$. For Partition II a second set of estimation equations are used, where the modified estimator of σ_1^2 is:

$$\widehat{\sigma}_1^2 = \frac{c_2 Y'(P_{x_{12}}-P_{x2})Y}{tr(P_{x_{12}}-P_{x2})V_1} - \frac{c_2\varepsilon_1 tr(P_{x_{12}}-P_{x2})Y'(I-P_{x_{12}})Y}{tr(P_{x_{12}}-P_{x2})V_1 tr(I-P_{x_{12}})}. \qquad (4)$$

The coefficients c_1, d_1 and d_2 and the coefficients c_2 and ε_1 that are involved in (3) and (4), respectively are chosen to minimize the MSE of $\widehat{\sigma}_1^2$. For details of the two different estimation equations and the involved coefficients, see appendix A.

4 Data

In the analyzed F2 cross, two European wild boars were mated to eight Large White sows. Four F1 boars were then mated to 22 F1 sows, producing 191 recorded F2 offspring in 26 families. In our analysis, we examined a meat quality trait (reflectance value, EEL), which is affected by the halothane gene located on chromosome 6 at position 80.4 cM. One of the founder boars was heterozygote (Hal^N/Hal^n) for this gene whereas all other founders were homozygotes (Hal^N/Hal^N) for the same allele. Following Knott et al. (1998), we included sex, litter and slaughter weight as fixed effects in our analysis. Family was included as random effect. Twenty two markers were genotyped on chromosome 6 at: 0.0, 8.6, 36.6, 49.7, 50.5, 62.9, 79.2, 80.4, 83.7, 84.1, 84.8, 90.6, 95.4, 100.7, 101.9, 115.9, 116.7, 119.0, 120.2, 124.0, 127.0 and 170.9 cM.

5 Analysis

The standard method to analyze experimental intercrosses is a simple regression model (Haley and Knott 1992), which assumes that there is a large genetic variation between breeds and small variation within breeds. The halothane gene would not have been detected with this model (Andersson-Eklund et al. 1998), because there was only one copy of the Hal^n-allele among the founders.

The variance component QTL model (1) was fitted at every 5 cM. Variance components were estimated using both non-modified and modified Henderson's method 3 with Partition I and II. These were compared to REML estimates. REML gives both the variance component estimates and a likelihood profile curve along the chromosome. A likelihood ratio was calculated at each position as: $LR = -2(l_0 - l_1)$ where l_1 is the log-likelihood for (1) at a specific position and l_0 is the log-likelihood under the null hypothesis of no QTL (i.e. for model (1) with u_1 deleted). Approximations of the LR-curve were calculated by calculating the log-likelihood for : $\widehat{\sigma}_2^2$ and $\widehat{\sigma}_e^2$ estimated under the null hypothesis and $\widehat{\sigma}_1^2$ estimated with one of the modified Henderson's estimators. The approximated LR values were put equal to 0 for negative values of $\widehat{\sigma}_1^2$.

6 Results

6.1 Variance component estimates

A QTL scan was performed at every 5 cM along pig chromosome 6 for the meat quality trait EEL. The phenotype observations from the F2 individuals were approximately normally distributed (Figure 1). The REML and the two modified Henderson 3 estimators of σ_1^2 were similar for most positions (Figure 2). The estimates at the halothane gene (80 cM) for REML, modified Partition I and modified Partition II, respectively, were: 4.96, 4.32, 5.06. The modified Partition I estimators resulted in slightly lower estimates and the difference was greatest at the right end of the chromosome around 150 cM.

The non-modified Partition I estimates tended to be lower than the REML estimates, whereas the non-modified Partition II estimates tended to be higher than the REML estimates (Figure 3). Hence, there was no clear results which of the two partitions that was the superior one.

6.2 Likelihood ratio curve

The LR curve, obtained from fitting the variance component QTL model in (1) using REML, showed a peak at 80 cM (Figure 4). This position coincides with the location of the halothane gene. The log-likelihood under the null hypothesis was l_0 = -378.0, and the REML variance component estimates of the family and residual effects were: $\widehat{\sigma}_2^2$ = 0.94 and $\widehat{\sigma}_e^2$= 17.6.

Approximated LR curves were calculated for both the modified Partition I and II estimates of the QTL variance, which gave good approximations of the correct LR curve (Figure 4).

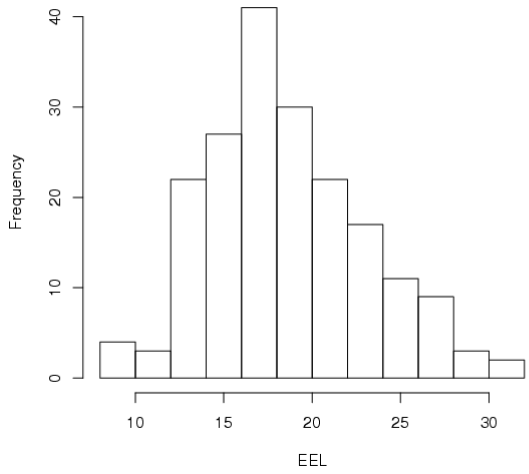

Figure 1: Histogram of the F2 individuals' meat quality (EEL) values from the studied Wild Boar x Domestic Pig intercross.

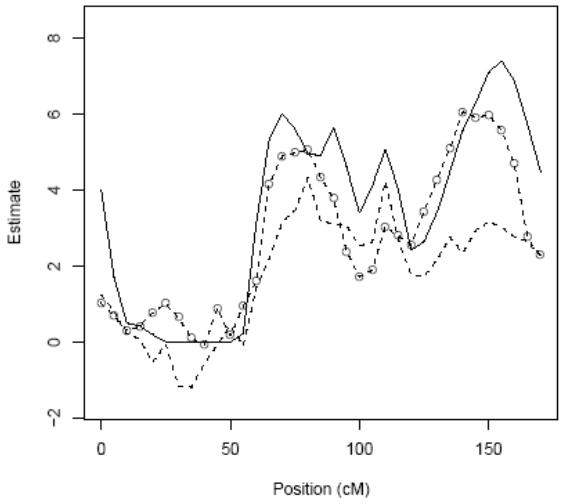

Figure 2: Estimates of the QTL variance σ_1^2 along pig chromosome 6. Solid line-REML estimates, dashed line-modified Partition I, dashed line with open circles-modified Partition II

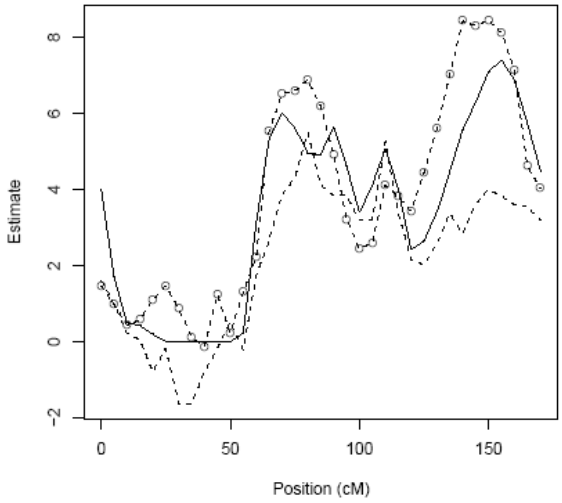

Figure 3: Non-modified estimators of the QTL variance σ_1^2 along pig chromosome 6. Solid line-REML estimators, dashed line-Partition I, dashed line with open circles-Partition II

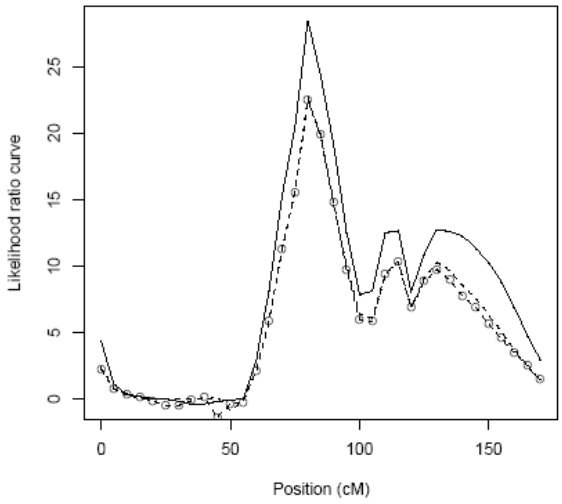

Figure 4: Likelihood ratio (LR) values along pig chromosome 6. The halothane gene affecting meat quality is located at 80 cM. LR values from REML given as solid line, approximated LR values from modified Partition I given as dashed line, and, approximated LR values from modified Partition II given as dashed line with open circles. The estimators from Partition I and II are very similar.

7 Discussion

We have tested a new non-iterative variance component estimation method on a QTL chromosome scan of the meat quality trait EEL. The variance component estimates differed substantially from REML estimates at several chromosome positions, but they were very close to the REML estimates at the QTL position. Moreover, the likelihood ratio curve could be very well approximated from our non-iterative VC estimators. Our method would also have given the same estimated position of the halothane gene as REML.

The large computational requirements of iterative REML algorithms are a major concern in QTL analysis (Rönnegård et al. 2007) and limits the analysis of large data sets. Furthermore, as the cost for genotyping decreases, the size of the analyzed pedigrees is likely to increase in the future, making full genome scans computationally slow or even infeasible. Our explicit solutions for the estimation of the QTL variance opens up new possibilities to develop fast and accurate QTL genome scan methods. The most computationally demanding part of the iterative REML algorithms is to calculate the inverse of V in each iteration. In the modified Partition I method, for instance, the only matrix inversions required are the generalized inverses of $(X, Z_1, Z_2)'(X, Z_1, Z_2)$, $(X, Z_1)'(X, Z_1)$ and $(X'X)$ in P_x, P_{x_1} and $P_{x_{12}}$, see Appendix A. These matrix inversions are relatively easy to optimize in computational speed when there are few columns in (X, Z_1, Z_2). The number of fixed effects are usually small in QTL problems, and the rank of the IBD matrices is either small or can be approximated with lower rank matrices (Rönnegård and Carlborg 2007, Rönnegård et al. 2007). We can, therefore, expect that (X, Z_1, Z_2) has few columns. Hence, our method should be easy to optimize numerically for two reasons; it is non-iterative and does not involve inverses of large matrices.

In conclusion, we have developed a novel method for QTL analysis, which is simpler to calculate than REML and gives better estimators than those obtained from Henderson's (1953) method.

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APPENDIX A:

Expressions for the reduction sum of squares needed for Henderson's method 3.

To estimate the VC of the model (1) we define the following matrices $[X]$, $[X, Z_1]$ and $[X, Z_1, Z_2]$. The corresponding projection matrices are

$$\begin{aligned} P_x &= X(X'X)^- X' \\ P_{x_1} &= (X, Z_1)((X, Z_1)'(X, Z_1))^-(X, Z_1)' \\ P_{x_{12}} &= (X, Z_1, Z_2)((X, Z_1, Z_2)'(X, Z_1, Z_2))^-(X, Z_1, Z_2)' \end{aligned}$$

where $-$ represents the g-inverse $AA^-A = A$. The first set of estimation equation Partition I are based on

$$\begin{cases} \mathrm{R}(u_1/\beta) \\ \mathrm{R}(u_2/\beta, u_1) \\ \mathrm{SSE} \end{cases}$$

where R(.) denote the reduction sum of squares and the residual sum of squares is denoted by SSE= $Y'(I - P_{x_{12}})Y$, see Searle (1971). The obtained variance component estimator from Partition I is

$$\begin{aligned} \widehat{\sigma}^2_{u_1} &= \frac{Y'(P_{x_1} - P_x)Y}{tr((P_{x_1} - P_x)V_1)} - \frac{tr((P_{x_1} - P_x)V_2)Y'(P_{x_{12}} - P_{x_1})Y}{tr((P_{x_1} - P_x)V_1)tr((P_{x_{12}} - P_{x_1})V_2)} \\ &\quad + \frac{kY'(I - P_{x_{12}})Y}{tr((P_{x_1} - P_x)V_1)tr((P_{x_{12}} - P_{x_1})V_2)tr(I - P_{x_{12}})} \end{aligned} \tag{5}$$

For the second set of estimation equations we need to define $[X, Z_2]$ and the corresponding projection matrix

$$P_{x_2} = (X, Z_2)((X, Z_2)'(X, Z_2))^-(X, Z_2)'$$

The second set of estimation equations Partition II are based on

$$\begin{cases} \mathrm{R}(u_1/\beta, u_2) \\ \mathrm{SSE} \end{cases}$$

The obtained variance component estimator from Partition II is

$$\begin{aligned} \widehat{\sigma}^2_1 &= \frac{\mathrm{tr}(I - P_{x_{12}})Y'(P_{x_{12}} - P_{x_2})Y - \mathrm{tr}(P_{x_{12}} - P_{x_2})Y'(I - P_{x_{12}})Y}{\mathrm{tr}(P_{x_{12}} - P_{x_2})V_2 tr(I - P_{x_{12}})} \\ &= \frac{Y'(P_{x_{12}} - P_{x_2})Y}{\mathrm{tr}(P_{x_{12}} - P_{x_2})V_1} - \frac{\mathrm{tr}(P_{x_{12}} - P_{x_2})Y'(I - P_{x_{12}})Y}{\mathrm{tr}(P_{x_{12}} - P_{x_2})V_1 \mathrm{tr}(I - P_{x_{12}})}. \end{aligned} \tag{6}$$

For the coefficients c_1, d_1 and d_2 given in (3) we have the following values

$$\begin{aligned} c_1 &= \frac{1}{\frac{2}{[tr(P_{x1}-P_x)V_1]^2}[tr(P_{x1}-P_x)V_1(P_{x1}-P_x)V_1]+1}, \\ d_1 &= \frac{1}{\frac{2}{[tr(P_{x_{12}}-P_{x1})V_2]^2}[tr(P_{x_{12}}-P_{x1})V_2(P_{x_{12}}-P_{x1})V_2]+1}, \\ d_2 &= \frac{\frac{(tr(P_{x1}-P_x)V_2)}{tr(P_{x_{12}}-P_{x1})V_2}d_1 tr(P_{x_{12}}-P_{x1})-tr(P_{x1}-P_x)}{[\frac{k}{tr(P_{x_{12}}-P_{x1})V_2}][\frac{2}{tr(I-P_{x_{12}})}+1]}. \end{aligned}$$

where $V_1 = Z_1 Z_1'$ and $V_2 = Z_2 Z_2'$. For details and calculations (see Al-Sarraj and von Rosen 2007).

Now for the coefficients that are involved in Partition II, i.e., c_2 and ε_1 given in (4) we refer to Kelly and Mathew (1994). However, we have calculated the values such that they would be appropriate for the second set of estimation equations Partition II,

$$\begin{aligned} c_2 &= \frac{1}{\frac{2}{[tr(P_{x_{12}}-P_{x2})V_1]^2}[tr(P_{x_{12}}-P_{x2})V_1 tr(P_{x_{12}}-P_{x2})V_1]}, \\ \varepsilon_1 &= \frac{1}{\frac{2}{tr(I-P_{x_{12}})}+1}. \end{aligned}$$