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Analysis of factorial experiments using mixed-effects models: options for estimation, prediction and inference

Razaw Al-Sarraj

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Razaw Al-Sarraj
Faculty of Natural Resources and Agricultural Sciences
Department of Energy and Technology
Uppsala

SLU
SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Abstract

In linear mixed-effects modelling of experiments, estimation of variance components, prediction of random effects, and computation of denominator degrees of freedom associated with inference on fixed effects, are important elements of the analysis. This thesis investigates alternatives to the likelihood-based procedures for analysis of factorial experiments with normally distributed observations. Consistent methods, such as the maximum likelihood method, can be disadvantageous in cases where only small samples are available. Moreover, the algorithms used in linear mixed-effects models can be computationally demanding in large datasets.

In this thesis, Henderson's method 3, a non-iterative variance component estimation method, was considered for estimation of the variance components in a two-way mixed linear model with three variance components. The variance component estimator corresponding to one of the random effects was improved by perturbing the standard unbiased estimator. The improved variance component estimator performed better in terms of mean square error.

In an application on a quantitative trait loci (QTL) study, the modified estimator was compared to the restricted maximum likelihood estimator on data from European wild boar × domestic pig intercross. The modified estimator was shown to approximate the results obtained from the restricted maximum likelihood (REML) method very closely.

For balanced and unbalanced data in two-way with and without interaction models, the generalized prediction intervals for the random effects were derived. The coverage probabilities of the proposed intervals were compared with those based on the REML method and the approximate methods of Satterthwaite (1946) and Kenward and Roger (1997). The coverage of the proposed intervals was closer to the chosen nominal level than coverage of prediction intervals based on the REML method.

With focus on Type I error, the implications of the available options in the `mixed` procedure of `SAS` and the `lmer` function of `R` for the inference on the fixed effects were examined. With the default setting of `SAS`, the frequency of Type I error was higher than with `R`. The Type I error rate in `SAS` was close to the nominal value when negative estimates of the variance components were allowed. Both software packages occasionally produced inaccurate results.

Keywords: generalized prediction intervals, Henderson's method 3, mean square error, mixed-effects models, QTL, REML, variance components

Author's address: Razaw Al-Sarraj, SLU, Department of Energy and Technology, P.O. Box 7032, 750 07 Uppsala, Sweden
E-mail: Razaw.Al-Sarraj@slu.se

Statistisk analys med linjära blandade modeller: alternativ för analys av faktoriella försök

Sammanfattning

När linjära blandade modeller används för statistisk analys av experiment är skattning av varianskomponenter, prediktion av slumpmässiga effekter och beräkning av frihetsgrader väsentliga delar av analysen. I denna avhandling undersöker vi alternativ till de likelihood-baserade metoder som oftast används vid analys av flerfaktoriella försök med normalfördelade observationer när modellen innehåller flera varianskomponenter. Asymptotiskt väl fungerande metoder, såsom likelihood-baserade metoder, kan fungera mindre bra när stickproven är små. Vid stora datamängder kan algoritmerna dessutom vara beräkningskrävande.

Hendersons metod 3 är en icke-iterativ metod för att skatta varianskomponenter i en linjär blandad modell. Denna var utgångspunkten för artikel I. Modellen innehöll två faktorer med slumpmässiga effekter. Estimatorn för den ena varianskomponenten modifierades genom att göra avkall på väntevärdesriktighet. Den modifierade estimatorn ger lägre medelkvadratfel.

I artikel II tillämpades den modifierade estimatorn på en studie av quantitative-trait-loci (QTL) i data från en korsning av europeiska vildsvin med tamgris. Den modifierade estimatorn jämfördes sedan med estimatorn baserad på restricted-maximum-likelihood (REML). De båda metoderna gav likartade skattningar av varianskomponenterna, däremot den modifierade estimatorn är mindre beräkningskrävande.

Generaliserad statistisk inferens har använts i artikel III. Ekvationer för generaliserade prediktionsintervall har härletts för några balanserade och icke balanserade modeller med slumpmässiga effekter. Täckningsgraden för de generaliserade prediktionsintervallen har jämförts med såväl täckningsgraden för REML-baserade prediktionsintervall som de approximativa prediktionsintervallen beräknade enligt Satterthwaites och Kenward och Rogers approximativa metoder. De generaliserade prediktionsintervallens täckningsgrader låg betydligt närmare den valda nominella nivån än vad de REML-baserade prediktionsintervallens täckningsgrader gjorde.

I artikel IV studerades proceduren `mixed` i `SAS` och funktionen `lmer` i `R`. Speciellt studerades olika alternativ som användaren kan välja mellan och valets betydelse för slutsatserna vad gäller modellens fixa effekter. Med de förvalda alternativen i `SAS` var frekvensen av typ-I fel högre än i `R`. När negativa variansskattningar tilläts i `SAS` låg frekvensen för typ-I-fel nära den valda nominella nivån. Ibland gav båda programvarupaketen helt felaktiga resultat.

Nyckelord: blandade modeller, generaliserade prediktionsintervall, Hendersons metod 3, medelkvadratfel, QTL, REML, varianskomponenter

Författarens adress: Razaw Al-Sarraj, SLU, Institutionen för energi och teknik, Box 7032, 750 07 Uppsala, Sverige

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Al-Sarraj, R. and von Rosen, D. (2009). Improving Henderson's method 3 approach when estimating variance components in a two-way mixed linear model. Peer reviewed book chapter in: *Statistical Inference, Econometric Analysis and Matrix Algebra.* Schipp, B. and Kräer, W. (Eds.). Physica-Verlag, Heidelberg, 125–142.

II. Rönnegård, L., Al-Sarraj, R. and von Rosen, D. (2008). Non-iterative variance component estimation in QTL analysis. *Journal of Animal Breeding and Genetics*, 126, 110–116.

III. Al-Sarraj, R., von Brömssen, C. and Forkman, J. (2018). Generalized prediction intervals for treatment effects in random-effects models. *Biometrical Journal*, 61:1242–1257.

IV. Al-Sarraj, R. and Forkman, J. (2020). Notes on correctness of p-values when analyzing experiments using SAS and R. *Unpublished manuscript.*

Papers I, II and III are reproduced with the permission of the publishers. Supplementary materials for Paper IV can be provided upon request.

The contribution of Razaw Al-Sarraj to the papers included in this thesis was as follows:

I. Planned the paper together with the co-author. Main responsibility for completing the manuscript with regular input and support from supervisors.

II. Planned the paper together with the author. Main responsibility for the method part of the paper.

III. Planned the paper together with the co-authors. Main responsibility for completing the manuscript with regular input and support from supervisors.

IV. Planned the paper together with the co-author. Main responsibility for completing the manuscript with regular input and support from supervisors.

1 Introduction

Over the past decades, the mixed linear models, also known as variance component models, have been a widely used tool for statistical analysis and inference in agricultural, medical, ecological, and environmental researches as well as in econometrics and social sciences. Mixed linear models comprise both fixed and random effects, consisting as such not only of the error variance component, but also of other variance components that are associated with the random effects. The fixed effects are estimated by the best linear unbiased estimation (BLUE) whereas the random effects are predicted by the best linear unbiased prediction (BLUP) (Henderson, 1975; Robinson, 1991; Searle et al., 1992).

Historically, the analysis of variance (ANOVA) has been the most common method used, especially in the agricultural experiments. The analysis and inference of the effects, fixed or random, initially require the estimation of the variance components. With ANOVA, the estimators are obtained through equating the observed and expected mean squares and subsequently solving the resulting equations for the estimators. For balanced data, the ANOVA method is known to have appealing properties, such as unbiasedness and minimum variance, among all unbiased estimators that are quadratic functions of the observations, (Graybill and Hultquist, 1961). In unbalanced data the situation is however more complicated, as all optimal properties lack to hold apart from unbiasedness.

Analogous to the balanced case, Henderson (1953) developed techniques suitable for all kinds of unbalanced data (Searle et al., 1992). With its three different variations, the method has been widely used in different areas of research, particularly in animal breeding. Other methods of estimation have been developed for both balanced and unbalanced data, with the likelihood-based methods being the most important ones, namely the maximum likelihood (ML) (Hartley and Rao,

1967), and the restricted maximum likelihood (REML) methods (Patterson and Thompson, 1971).

The methods vary in their degree of computational effort. In the ANOVA method, the estimators are obtained explicitly, whereas the likelihood-based methods are computationally extensive. Despite the availability of technologies nowadays, there are research problems demanding a huge number of computations, for instance in image analysis and in animal breeding experiments involving quantitative trait loci (QTL) analysis. The ANOVA-method can yield negative estimates of the variance components, which in the likelihood-based methods can be constrained at zero.

The likelihood-based methods are well established in most software programmes. For instance, the REML method is often set as the default option for estimation of variance components. This is the case for both the `mixed` procedure of `SAS` (Stroup et al., 2018) and the `lmer` function of `R` (Bates et al., 2015). In practice, estimates are often bounded to be non-negative, however, zero-estimates of the variance components may lead to problems with regard to certain aspects of the analysis. For instance, the F-tests employed for testing the fixed-effects hypothesis are in general approximate and the number of degrees of freedom in the denominator may be affected by the zero-estimate of the variance component. Different methods for estimating the denominator degrees of freedom are available (Verbeke and Molenberghs, 2000). The Kenward and Roger (1997, 2009) method has been recommended by many authors (Alnosair, 2007; Chen and Wei, 2003; Spilke et al., 2005a,b). However, this method is implemented differently in different softwares when a zero-estimate is computed for the variance component, sometimes resulting in different, or even incorrect, conclusions.

In the analysis of agricultural field experiments, variance components are employed to quantify the variation between the experimental units. In a simple randomized complete block experiment with a block variance and an error variance component, a zero-estimate of the block variance may result in the neglecting of the block-effects. Hence, the analyses of the experiment would proceed as if no blocks existed. Similarly, for a split-plot experiment with main plot-, sub-plot- and error variance components, a zero-estimate of the main-plot variance component would result in the main plot effect being neglected.

Furthermore, a zero-estimate of the variance component may affect the prediction of the random effects, i.e. the BLUPs, and may even impact their prediction intervals. In a model with random effects of ex-

perimental treatments, the BLUPs are characterized by their shrinkage property (James and Stein, 1961). In a simple one-way random-effects model, all BLUPs become equal to the overall mean when the between-treatment variance is zero (Yu et al., 2015). Noted by Forkman and Piepho (2013), the prediction intervals for the random effects degenerate at zero when the between-treatments variance in a randomized complete block experiment is estimated to zero. In order to overcome the problem of inavailability of classical confidence intervals for a parameter of interest, the generalized inference methodology introduced by Tsui and Weerahandi (1989) and Weerahandi (1993) can be employed.

Taking into consideration the difficulties encountered with the available methods, there is a need to modify and explore alternatives to the already existing methods.

1.1 Aim and outline of the thesis

The general aim was to explore alternatives to likelihood-based procedures for analysis of factorial experiments using linear mixed-effects models. If only small samples are available, using an asymptotic approach such as the likelihood-based methods can be misleading. Moreover, the algorithms used in linear mixed models can be computationally demanding. With explicit estimators, it is possible to speed up computations. The thesis includes four papers:

i) Improving Henderson's method 3 approach when estimating variance components in a two-way mixed linear model

ii) Non-iterative variance component estimation in QTL analysis

iii) Generalized prediction intervals for treatment effects in random-effects models

iv) Notes on correctness of p-values when analyzing experiments using `SAS` and `R`

In Paper I, the intention was to improve Henderson's method 3 for a two-way linear mixed-effects model with three variance components, to achieve a better performance in terms of mean square error.

In Paper II, the modified variance component estimator of Paper I is applied on QTL data to investigate replacement of the REML method by a non-iterative estimation method.

In Paper III, generalized prediction intervals for random effects are derived and introduced in some balanced and unbalanced models intending to reach intervals with better coverages compared to those obtained from other methods.

In Paper IV, the implications of the available options in the software packages of `SAS` and `R` are investigated on accuracy of results using linear mixed-effects models. Furthermore, it is examined under which circumstances the software produce incorrect Type I error rates.

The outline of the thesis is as follows:

- Chapter 2 presents a background to the models and the estimation methods.
- Chapter 3 summarizes the papers.
- Chapter 4 provides a general discussion with conclusions.
- The four papers, which are the basis for the thesis, are provided in the Appendix.

2 Theory and methods

This chapter presents the fixed-, random- and mixed-effects models as well as the inference on the fixed effects and the methods used for variance component estimation. Henderson's three methods for variance component estimation are described in general with the focus on the third method (Searle et al., 1992). Finally, the generalized confidence intervals are presented (Weerahandi, 1993, 1995).

2.1 Fixed-effects models

A general fixed-effects linear model has been described by many authors, among others Searle (1971) and Graybill (1976). Let

$$Y = X\beta + e, \tag{1}$$

where $Y : N \times 1$ is a vector of observations, $X : N \times m$ is the design or incidence matrix of known elements and β: $m \times 1$ is a vector of unknown fixed-effects parameters. Finally, e: $N \times 1$ is a vector representing the within-subject variability or the measurement error, assumed to have mean 0 and dispersion matrix $\sigma_e^2 I_N$, where I_N is the $N \times N$ identity matrix. This implies that the expected value of Y is $\mathrm{E}(Y) = X\beta$ and its dispersion matrix is $\mathrm{D}[Y] = \sigma_e^2 I_N$.

If X is of full rank, then the best linear unbiased estimator (BLUE) is obtained from the least squares solution for the fixed effects β is $\hat{\beta} = (X'X)^{-1}X'Y$. Otherwise, if X is not of full rank, i.e. some columns are linearly dependent, then the model is overparameterized and $(X'X)^{-1}$ cannot be obtained. Instead, a generalized inverse, $(X'X)^{-}$, can be employed and the solution for β is $\hat{\beta} = (X'X)^{-}X'Y$ (Searle, 1971). This solution is not unique. But unique solutions can be computed for the so-called estimable functions, which are linear functions of the parameters.

Assume e is an independently normally distributed random variable, $e \sim N(0, \sigma_e^2 I_N)$. Then Y is distributed as $Y \sim N(X\beta, \sigma_e^2 I_N)$. A general form for the null hypotheses about the fixed effects in (1) can be expressed as

$$H_0 : L\beta = 0, \tag{2}$$

where L is a row vector or matrix corresponding to a linear function of X. The null hypothesis (2) can be tested using the F-statistic

$$F = \frac{Q_A/k}{\hat{\sigma}_e^2}, \tag{3}$$

where $Q_A = (L\hat{\beta})'(L(X'X)^{-}L')^{-1}(L\hat{\beta})$, $\hat{\sigma}_e^2$ is the estimated error variance, and k is the degrees of freedom which is equal to the row rank of L, i.e. $k = \text{rank}(L)$ (the rank of a matrix, is the number of linearly independent rows or columns of the matrix). Under the null hypothesis, F in (3) is F-distributed with k an $N - \text{rank}(X)$ degrees of freedom.

Alternatively, the problem of overparameterized models can be dealt with by laying constraints on the levels of the factors in the model. This is usually performed by setting the parameters corresponding to the first or last level to zero, or simply constrain the factor levels to add up to zero, which is known as sigma-restriction (Searle, 1987).

2.2 Mixed-effects models

Model (1) may be extended by adding a random term, often denoted in the literature by Zu,

$$Y = X\boldsymbol{\beta} + Zu + e, \tag{4}$$

where the terms Y, X and β are defined as in (1). The term Zu given in model (4) can be partitioned conformably as

$$Zu = \begin{bmatrix} Z_1 & Z_2 & \dots & Z_r \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \\ \vdots \\ u_r \end{bmatrix} = \sum_{i=1}^{r} Z_i u_i.$$

Thus, model (4) can be rewritten as

$$Y = X\boldsymbol{\beta} + \sum_{i=1}^{r} Z_i u_i + e. \tag{5}$$

The new added matrix Z_i is an $N \times n_i$ incidence matrix of known elements, u_i is an $n_i \times 1$ vector of random effects, $i = 1, \ldots, r$, with expected value $\mathrm{E}(u_i) = 0$ and dispersion matrix $\mathrm{D}[u] = G$. The error term, e, is an $N \times 1$ random vector with $\mathrm{E}(e) = 0$ and dispersion matrix $\mathrm{D}[e] = R$. Further it is assumed that u_i and e are uncorrelated random variables. It follows that $Y \sim (X\boldsymbol{\beta}, V)$, where $\mathrm{E}(Y)$ is $X\beta$ and the dispersion matrix, V, is $ZGZ' + R$. Model (4), is referred to as a mixed model (Searle et al., 1992; Stroup et al., 2018).

An important special case of model (4) is when the only fixed effect in model is the overall mean, μ. In this case, the model can be written as

$$Y = X\mu + Zu + e, \tag{6}$$

where $X = 1_N$ is an N-vector of ones, μ is a scalar and all other components (Y, Z, u, e) are defined as in model (4). This model is referred to as a random-effects model.

Inference on fixed-effects

In model (4), let the random variables u and e be independently and normally distributed, $u \sim N(0, G)$ and $e \sim N(0, R)$. It follows that $Y \sim N(X\beta, V)$, where $V = ZGZ' + R$. Usually, the matrices G and R are unknown and have to be estimated from the observed data. The estimates are denoted as $\hat{G}$ and $\hat{R}$, respectively. Henderson (1975) developed a set of estimation equations, referred to as Henderson's mixed model equations, which simultaneously yield the BLUE of $X\boldsymbol{\beta}$ and the BLUP of the random effects u. The solutions of Henderson's mixed model equations are

$$\hat{\beta} = (X'\hat{V}^{-1}X)^{-1}X'\hat{V}^{-1}Y,$$

and

$$\hat{u} = \hat{G}Z'\hat{V}^{-1}(Y - X\hat{\beta}),$$

where $\hat{V} = Z\hat{G}Z' + \hat{R}$. The test statistic for testing the fixed effects hypothesis in (2) can be expressed as

$$F = \frac{(L\hat{\boldsymbol{\beta}})'(L(X'\hat{V}^{-1}X)^{-}L')^{-1}(L\hat{\boldsymbol{\beta}})}{k}. \tag{7}$$

This F-statistic follows an approximate F distribution, with numerator degrees of freedom k equal to the rank of L and approximate denominator degrees of freedom that can be estimated using various

methods. Among them, the Satterthwaite (1946) and the Kenward and Roger (1997) methods are the most commonly used (Verbeke and Molenberghs, 2000).

2.3 The different types of sums of squares

At least three types of sums of squares are used to calculate the numerator of the F-tests given in (3) and (7). In the major statistical software packages of e.g. the `mixed` procedure of `SAS` (Stroup et al., 2018) and the `lmer` function of `R` (Bates et al., 2015), they are referred to as Type I, Type II and Type III sums of squares. When data is balanced, all three are equivalent. For unbalanced data they are not. All three methods were introduced in a landmark paper by Yates (1934). In that paper, Yates' unadjusted and adjusted method of fitting constants, and the method of weighted squares of means, correspond to the Types I, II and III sums of squares, respectively.

Consider a two-way fixed-effects model with interaction.

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + e_{ijk}, \tag{8}$$

where $i = 1, \ldots, a$, $j = 1, \ldots, b$, and $k = 1, \ldots, n_{ij}$. Here, y_{ijk} is the response of the kth observation on the ith level of factor A and the jth level of factor B, μ is the overall mean and α and β are unknown fixed effects of the factors A and B, respectively. The interaction effect between the i-th and j-th level of the two factors A and B is denoted as γ_{ij}. The experimental errors are assumed to be independently and normally distributed, $e_{ij} \sim N(0, \sigma_e^2)$. The data for such a model can be represented in a table consisting of a columns and b rows and n_{ij} observations in the ij-th cell, and assuming $n_{ij} > 0$ for every i and j.

Model (8) can be rewritten as

$$y_{ijk} = \mu_{ijk} + e_{ijk}, \tag{9}$$

where $\mu_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij}$ is the expected value in the ij-th cell. Table 1 illustrates the layout of a two-way factorial experiment with two levels of factor A and three levels of factor B,

Table 1: Two-way factorial experiment layout

	Factor A		
Factor B	1	2	Total
1	$\mu_{11}(n_{11})$	$\mu_{21}(n_{21})$	$\mu_{.1}(n_{.1})$
2	$\mu_{12}(n_{12})$	$\mu_{22}(n_{22})$	$\mu_{.2}(n_{.2})$
3	$\mu_{13}(n_{13})$	$\mu_{23}(n_{23})$	$\mu_{.3}(n_{.3})$
Total	$\mu_{1.}(n_{1.})$	$\mu_{2.}(n_{2.})$	$\mu_{..}(n_{..})$

where $n_{..} = \sum_{j=1}^{a} \sum_{j=1}^{b} n_{ij}$ is the total number of observations, and the number of observations for the levels of factor A and factor B are $n_{i.}$ and $n_{.j}$, respectively. We let $\mu_{i.}$ and $\mu_{.j}$ denote the marginal means for factor level i averaged over all the levels of factor j, and for factor level j averaged over all the levels of factor i, respectively.

For instance, consider testing the hypothesis for the main factor A in model (8). The three types of sums of squares can be expressed as follows:

- Type I test: a sequential type of sums of squares. It is calculated by adjusting each effect to the one that has emerged in the model before that term. The null hypothesis for the main factor A when factor A enters the model first, is

$$H_0 : (1/n_{1.}) \sum_{j=1}^{b} n_{1j}\mu_{1j} = \cdots = (1/n_{a.}) \sum_{j=1}^{b} n_{aj}\mu_{aj}, \tag{10}$$

  where $n_{i.}$ is the total number of observations for i-th level of factor A. Each cell mean μ_{ij} is weighted with respect to the number of observations in the ij-th cell.

- Type II test: the sum of squares for each effect are adjusted for the other effects except for the interaction. The null hypothesis tested by this type is

$$H_0 : \sum_{j=1}^{b} n_{ij}\mu_{ij} = \sum_{k=1}^{a} \sum_{j=1}^{b} (n_{ij}n_{kj}/n_{.j})\mu_{kj} \quad \text{for all} \quad i, j, i \neq k, \tag{11}$$

  where $n_{.j}$ is the total number of observations of the j-th level of factor B.

- Type III test: each effect is adjusted for the other effects. This test is most easily performed if the parameters are constrained to add up to zero when summed over any subscript, i.e. if the sigma-restricted parametrization is used (Searle, 1987). The hypothesis tested is

$$H_0 : \mu_{1.} = \mu_{2.} = \cdots = \mu_{a.}, \tag{12}$$

where $\mu_{i.} = \sum_{j=1}^{b} \mu_{ij}/b$ is the unweighted marginal mean of the i-th treatment. All cells are weighted the same, regardless of their cell frequencies.

Applying the three different types of sums of squares on model (8), provides the same p-value for the interaction. The main difference between the sums of squares when data is unbalanced, comes down to what hypotheses is being tested (Pendleton et al., 1986; Speed et al., 1978). In general, null hypotheses should be specified before conducting the experiment. If observations are missing at random, this cannot be done using (10) and (11).

2.4 A review of variance component estimation methods

When mixed models are considered, there are many methods for estimation of variance components. The most commonly used methods are

i) the ANOVA-based methods, (Henderson, 1953),

ii) the likelihood-based methods, such as the ML and REML methods (Hartley and Rao, 1967; Harville, 1977; Patterson and Thompson, 1971), and

iii) the minimum norm quadratic unbiased estimation (MINQUE) and minimum variance quadratic unbiased estimator (MIVQUE) methods (Rao, 1970, 1971a,b, 1972).

The ANOVA method is the oldest procedure used in the biological sciences. It was pioneered by Fisher (1918) while developing the theory of quantitative genetics to describe the inheritance of continuous traits. Despite the fact that many others followed, they all dealt with the balanced data case; see the book by Searle et al. (1992) for a comprehensive literature review. It was first in Cochran (1939) that unbalanced data appeared and the principle of equating sums of squares to their expectations was used on a one-way random model, which is a special

case of the model defined in (6). The paper by Henderson (1953) addressed the problem of variance component estimation in unbalanced data. This paper has played an important role, among others in population genetics and in animal breeding, where the use of estimates of variance components is important for the application of selection theory. However, the method leads to negative estimates of the variance components with positive probability. Therefore, it has been essential to develop other estimation procedures.

Hartley and Rao (1967) introduced the ML procedure to variance component estimation. This is a likelihood-based estimation procedure, i.e. it assumes a probability distribution for the underlying data. It maximizes the full likelihood function over the parameter space. Patterson and Thompson (1971) proposed the REML procedure. This method has been reviewed extensively by Harville (1977). In the REML procedure, the likelihood under normality is partitioned into two parts; one being free of the fixed effects. Maximizing this part over the optionally non-negative space of variance components parameters, yield the REML estimators. These two likelihood-based procedures take care of the deficiency of the ANOVA-based method since positive estimators can be obtained. They also have several nice statistical properties such that they are functions of the sufficient statistics, consistent, and asymptotically normal. Furthermore, the asymptotic sampling dispersion matrix of the estimators is known.

Rao (1971a,b, 1972) proposed a general method for estimating a linear function of the variance components by a quadratic function of the data. This method is called MINQUE. According to this method, the estimators of the variance components can be obtained explicitly and the method is distribution free. This is of advantage, in comparison to the ML and REML procedures. The most important property of the MINQUE method is unbiasedness. However, the method can produce negative estimates. All the methods mentioned above vary in the degree of their computational effort. For the ANOVA and the MINQUE method, the estimators can be obtained explicitly, while the likelihood-based methods require a numerical solution. In other words, the ML and REML estimators require iterative procedures to solve the set of non-linear estimation equations, see Searle et al. (1992) for details.

Depending on which method is used, different variance component estimates are obtained. How to choose between them has been an issue of great importance. Therefore, some authors have compared the

performance of various estimators with each other in terms of some criteria, e.g. squared bias and mean square error, using simulation techniques. Swallow and Monahan (1984) compared different estimation procedures for a one-way random model with a between-group variance (σ_1^2) and an error variance (σ_e^2). Among other methods, the ANOVA, MIVQUE, REML and ML methods were compared in terms of MSE and bias. This was carried out by a Monte Carlo simulation under several data layouts and for several values of σ_1^2/σ_e^2. According to their results, the ANOVA method was adequate and the estimators performed well except with seriously unbalanced data when $\sigma_1^2/\sigma_e^2 > 1$. In addition, when $\sigma_1^2/\sigma_e^2 < 0.5$, the performance of the estimator σ_1^2 obtained by ML was better than the ones obtained using the other methods.

For the same model and data layouts of Swallow and Monahan (1984), Conerly and Webster (1987) considered the MSE of the MINQE (Rao and Chaubey, 1978), i.e. the MINQUE without the condition of unbiasedness, and compared it with the MSE of the estimators investigated by Swallow and Monahan (1984). When $\sigma_1^2 > \sigma_e^2$, the MSE of the estimator σ_1^2 obtained using the MINQE method was smaller than the MSE of the estimators obtained using the other methods.

2.5 ANOVA-based methods for variance component estimation

Originally, the ANOVA-based methodology was used to estimate the variance associated with the error component, σ_e^2, in models with balanced data, where all factors' levels were considered fixed. The estimator was obtained through equating the expected mean square errors (or the sums of squares) to the corresponding value calculated from the data, i.e. E(MSE) = MSE. Thus,

$$\mathrm{E(MSE)} = \sigma_e^2,$$

yielding the following variance estimate $\hat{\sigma}_e^2 = \mathrm{MSE}$.
This idea was extended to models with several variance components. Let σ^2 be the vector of variance components to be estimated in some model, and let s be a vector of the sums of squares. Further, let C be a non-singular matrix. Then taking the expected value

$$E(s) = C\sigma^2, \tag{13}$$

yields the ANOVA estimator of σ^2, based on (13) and is the solution to $s = C\hat{\sigma}^2$. It follows that $\hat{\sigma}^2 = C^{-1}s$.

The expression in (13), can be extended to include not only sums of squares but also any set of quadratic forms. Let q be the $m \times 1$ vector of quadratic forms such that

$$E(q) = A\sigma^2, \tag{14}$$

where A is a $m \times r$ matrix of known coefficients and $\sigma^2 = (\sigma_1^2, \sigma_2^2, \ldots, \sigma_r^2)'$ is the vector of $r \times 1$ variance components. If A is square, i.e. $m = r$ and non-singular, then an unbiased estimator of σ^2 can be obtained, as in expression (13), from the solution $\hat{\sigma}^2 = A^{-1}q$. In certain situations when $m > r$, i.e. when there are more quadratic forms than there are variance components to estimate, the following can be used to obtain an unbiased estimator: $\hat{\sigma}^2 = (A'A)^{-1}A'q$ (Searle et al., 1992).

2.6 Henderson's three methods

In a landmark paper, Henderson (1953) presented three methods for estimation of variance components, suitable for unbalanced data layouts. The methods are known as Henderson's methods 1, 2 and 3. They are sometimes described as three different ways of using the general ANOVA-method (Searle, 1987). All three methods equate the expected mean square errors to their observed values, and subsequently solve the resulting equations to obtain the estimators of the variance components. Method 1 uses sums of squares and is a counterpart to the ones used in the analysis of variance for balanced data. This method is employed when random models are considered, i.e. models that apart from the overall mean, consist of only random components. Method 2 uses sums of squares after being corrected for the fixed effects in the model. Therefore, this type can be used on mixed models. However, it cannot be used on models with interactions between the fixed and random effects. Finally, method 3 uses various reductions in sums of squares due to fitting sub-models of the full model. The drawback with this method is that the obtained estimators are not unique since the method can give rise to more equations than there are variance components to be estimated. Method 3 can be employed on mixed models with or without interactions. All three methods yield unbiased estimators and are easy to compute. Furthermore, no distributional assumptions are required. However, the estimators of the variance components can assume negative values. When data is balanced, all three methods reduce to the customary ANOVA method. The merits and demerits

of the three methods are presented and discussed thoroughly in Searle (1968) and Searle et al. (1992).

Henderson's method 3 for two variance components

Henderson's method 3 uses reductions in sums of squares due to fitting sub-models of the full model. Thereafter, the estimated variance components are obtained by equating the reduced sums of squares to their respective expected values. For this purpose, the so-called R(.)-notation which was used by Searle (1971, 1987), can be employed. Let R(.) denote the reduction in the total sums of squares due to fitting a model. In other words, R(.|.) is the sum of squares due to regression. For simplicity, we illustrate the method with model (5), consisting of only one random effect (u_1), i.e. a model including two variance components, the first, σ_1^2, associated with the random effect u_1, and the second being the error variance component σ_e^2. The full model is

$$Y = X\beta + Z_1 u_1 + e. \quad \text{(full model)} \tag{15}$$

The reduction sums of squares due to fitting this model is denoted as $\mathrm{R}(\beta, u_1)$. A sub-model (reduced model) is

$$Y = X\beta + e. \quad \text{(sub-model)} \tag{16}$$

The reduction in sums of squares of this model is $\mathrm{R}(\beta)$. Thus, the difference in reduction is $\mathrm{R}(u_1|\beta) = \mathrm{R}(\beta, u_1) - \mathrm{R}(\beta)$. This represents the reduction sum of squares due to adding an extra term u_1 to the sub-model. Model (15) consists of two variance components, hence, one set of estimation equations may be considered:

$$\begin{cases} \mathrm{R}(u_1|\beta) \\ \mathrm{SSE} \end{cases} \tag{17}$$

where SSE denotes the error sum of squares. Each reduction can be written as $Y'X(X'X)^{-}X'Y$, where X is compatible with the considered model, and with $X(X'X)^{-}X'$ idempotent. Let $A = X(X'X)^{-}X'$, then each reduction in sums of squares can be expressed in the form, $Y'AY$, a quadratic form of the data for some symmetric matrix A. The estimates of the variance components can be obtained by equating the reductions in sums of squares to their expected values. We define the following partitioned matrices: $X = [X]$ and $X1 = [X, Z_1]$. The

reductions in sums of squares for models (15) and (16) can respectively be expressed as:

$$\begin{aligned} \mathrm{R}(\beta, u_1) &= Y'X_1(X_1'X_1)^{-}X_1'Y = Y'P_{x_1}Y, \\ \mathrm{R}(\beta) &= Y'X(X'X)^{-}X'Y = Y'P_xY, \end{aligned} \tag{18}$$

where $P_{x_1} = X_1(X_1'X_1)^{-}X_1'$ and $P_x = X(X'X)^{-}X'$ are projection matrices. A projection matrix for a matrix w can be defined as $P_w = w(w'w)^{-}w'$. Thus P_w is an idempotent matrix; for more properties see Schott (1997).

Then $\mathrm{R}(u_1|\beta)$ and SSE can be expressed as:

$$\begin{aligned} \mathrm{R}(u_1|\beta) &= Y'(P_{x_1} - P_x)Y, \\ \mathrm{SSE} &= Y'(I - P_{x_1})Y. \end{aligned} \tag{19}$$

The following result follow regarding the mean of $Y'AY$, see Searle (1971):

- Let $Y \sim N(\mu, V)$ and A be a symmetric matrix, the mean of $Y'AY$, is equal to

$$\mathrm{E}(Y'AY) = \mathrm{tr}(AV) + \mu'A\mu. \tag{20}$$

  The notation tr, i.e. the trace function for a square matrix, corresponds to the sum of the diagonal elements. The result in (20) is true regardless of whether Y is normally distributed or not. Moreover, when $\mu = 0$, then $\mathrm{E}(Y'AY) = \mathrm{tr}(AV)$.

For our computations, assume Y is normally distributed with mean $X\beta$ and dispersion $V = \sigma_1^2V_1 + \sigma_e^2I_N$ with $V_1 = Z_1Z_1'$. Thus, taking the expected value of (19), and equating it to its corresponding observed values, and solving the resulting equations, the variance components are obtained. Using the result in (20)

$$\begin{aligned} E[\mathrm{R}(u_1|\beta)] &= \mathrm{tr}(P_{x_1} - P_x)V, \\ E[SSE] &= \mathrm{tr}(I - P_{x_1})V, \end{aligned} \tag{21}$$

The set of equations may be arranged in a vector. Thereafter, by equating these expected values to their observed ones we get

$$\begin{bmatrix} Y'(P_{x_1} - P_x)Y \\ Y'(I - P_{x_1})Y \end{bmatrix} = J \begin{bmatrix} \sigma_1^2 \\ \sigma_e^2 \end{bmatrix},$$

where

$$J = \begin{bmatrix} \text{tr}(P_{x_1} - P_x)V_1 & \text{tr}(P_{x_1} - P_x)I \\ \text{tr}(I - P_{x_1})V_1 & \text{tr}(I - P_{x_1})I \end{bmatrix}.$$

The estimators of the variance components are

$$\begin{bmatrix} \widehat{\sigma}_1^2 \\ \widehat{\sigma}_e^2 \end{bmatrix} = J^{-1} \begin{bmatrix} Y'(P_{x_1} - P_x)Y \\ Y'(I - P_{x_1})Y \end{bmatrix}.$$

Note that $(P_{x_1} - P_x)$ and $(I - P_{x_1})$ are idempotent matrices. Moreover, since $P_{x_1}V_1 = V_1$, then $\text{tr}(I - P_{x_1})V_1 = 0$, the J matrix reduces to

$$J = \begin{bmatrix} \text{tr}(P_{x_1} - P_x)V_1 & \text{tr}(P_{x_1} - P_x)I \\ 0 & \text{tr}(I - P_{x_1})I \end{bmatrix}.$$

Thus,

$$\begin{bmatrix} \widehat{\sigma}_1^2 \\ \widehat{\sigma}_e^2 \end{bmatrix} = \begin{bmatrix} \text{tr}(P_{x_1} - P_x)V_1 & \text{tr}(P_{x_1} - P_x)I \\ 0 & \text{tr}(I - P_{x_1})I \end{bmatrix}^{-1} \begin{bmatrix} Y'(P_{x_1} - P_x)Y \\ Y'(I - P_{x_1})Y \end{bmatrix}.$$

Solving the above system of equations gives the variance component estimators

$$\widehat{\sigma}_1^2 = \frac{Y'(P_{x_1} - P_x)Y}{\text{tr}(P_{x_1} - P_x)V_1} - \frac{\text{tr}(P_{x_1} - P_x)Y'(I - P_{x_1})Y}{\text{tr}((P_{x_1} - P_x)V_1)(\text{tr}(I - P_{x_1}))}, \tag{22}$$

and

$$\widehat{\sigma}_e^2 = \frac{Y'(I - P_{x_1})Y}{\text{tr}(I - P_{x_1})}. \tag{23}$$

Henderson's method 3 for three variance components

Models consisting of more than two variance components have more than one estimation equation for estimating the variance components. For instance, in a two-way mixed model with no interaction there are three variance components to be estimated of which two are associated with the two random effects (σ_{u1}^2, σ_{u2}^2) and the third is the error variance component σ_e^2. Let $\text{R}(\beta, u_1, u_2)$ denote the reduction in sums of squares due to fitting the full model

$$Y = X\beta + Z_1u_1 + Z_2u_2 + e. \quad \text{(full model)} \tag{24}$$

Further, let $\text{R}(\beta)$, $\text{R}(\beta, u_1)$ and $\text{R}(\beta, u_2)$ be the reductions due to fitting the sub-models

$$
\begin{aligned}
Y &= X\beta + e, \\
Y &= X\beta + Z_1 u_1 + e, \\
Y &= X\beta + Z_2 u_2 + e,
\end{aligned} \tag{25}
$$

respectively. For model (24), two sets of estimation equations may be considered

$$
\begin{cases} \mathrm{R}(u_1|\beta) \\ \mathrm{R}(u_2|\beta, u_1) \\ \mathrm{SSE} \end{cases} \quad \text{or} \quad \begin{cases} \mathrm{R}(u_2|\beta) \\ \mathrm{R}(u_1|\beta, u_2) \\ \mathrm{SSE} \end{cases}
$$

Assuming normality, all the reductions in sums of squares follow a non-central χ^2 distribution, and all the reductions are independent of each other and of SSE, see Searle (1987).

If we consider, for instance, the left-hand side set of estimation equations in an analysis of variance context: the partitioning of the total sums of squares are as in Table 2.

Table 2: Analysis of variance

Source of Variation	Sum of squares
β	$\mathrm{R}(\beta)$
u_1 adjusted for β	$\mathrm{R}(u_1\|\beta) = \mathrm{R}(u_1, \beta) - \mathrm{R}(\beta)$
u_2 adjusted for β and u_1	$\mathrm{R}(u_2\|\beta, u_1) = \mathrm{R}(\beta, u_1, u_2) - \mathrm{R}(\beta, u_1)$
Error	SS_E

Of note, the quadratics in Table 2 are equivalent to using Type I sums of squares when u_1 enters the model first followed by u_2 (Searle et al., 1992).

2.7 Generalized confidence intervals (GCIs)

A classical confidence interval can be defined as follows: let $(y_1, y_2, \cdots, y_n)$ be a random sample from a random variable Y with known distribution function $f(Y|\upsilon)$, where $\upsilon = (\theta, \delta)$ are the unknown parameters, θ is the parameter of interest and δ is the nuisance parameter (a parameter not of interest). Let α be a confidence coefficient, i.e. a pre-specified constant between 0 and 1. Let $L = L(y_1, \cdots, y_n)$ and $U = U(y_1, \cdots, y_n)$ be two functions of the data such that

$$
P[L \leq \theta \leq U] = 1 - \alpha,
$$

where P denotes probability. Then $[L, U]$ is said to be a confidence interval for θ with $1 - \alpha$ confidence.

In general, confidence intervals can be computed using functions of the data, particularly when the distribution is free of nuisance parameters. However, even with the presence of a nuisance parameter, confidence intervals can be constructed using a function of both the data and the unknown parameters, the so-called pivotal quantity (pivot). A pivot can be defined as a random variable whose distribution is free of any unknown parameter, including the nuisance parameters (Pawitan, 2001).

The generalized concept introduced by Tsui and Weerahandi (1989) was used by Weerahandi (1993) to extend the standard notion of a pivotal quantity to a generalized pivotal quantity (GPQ). The GPQs can be used to construct confidence intervals. The obtained intervals are referred to as generalized confidence intervals (GCIs).

The method can be described as follows: suppose we want to construct a GCI for a scalar parameter θ. Let Y be an observable random variable whose distribution depends on unknown parameters (θ, δ), where θ is the parameter of interest and δ is a nuisance parameter (possibly a vector parameter). Let the observed value of Y be denoted as lower case y and let $G = G(Y; y, \theta, \delta)$ be a function of Y, y, θ, and δ. Then G is a GPQ for θ if it satisfies the following two properties:

> Property i: For a fixed y, the distribution of G does not depend on any of the unknown parameters.
>
> Property ii: The observed value of G, denoted as $g(y; y, \theta, \delta)$, is free of the nuisance parameters δ.

A two-sided equal-tailed $(1 - \alpha)$ GCI is given by

$$G_{\alpha/2} < \theta < G_{1-\alpha/2},$$

where G_α is the (100α)th percentile of the distribution of G. The quantiles can be estimated using Monte Carlo methods. Property i ensures that the confidence region does not depend on the parameters θ and δ. Property ii guarantees that the generalized confidence region can be constructed using only observed data.

The method described above can be used to compute confidence intervals for the parameters in random- and mixed-effects models. In addition, the method is applicable to various interval estimation problems where the standard methods are difficult to apply. Many applications appear in Weerahandi (1993, 1995).

Example: GCI for σ_A^2

Consider a one-way random-effects model with balanced data,

$$Y_{ij} = \mu + u_i + e_{ij}, \quad i = 1, \ldots, a; \quad j = 1, \ldots, n, \tag{26}$$

where u_i and e_{ij} are independent random variables with $u_i \sim N(0, \sigma_A^2)$ and $e_{ij} \sim N(0, \sigma_e^2)$, a is the number of groups, and n is the number of observations per group. Let X_A and X_E denote the between groups and error sums of squares, respectively. The ANOVA for model (26) is as shown in Table 3.

Table 3: ANOVA for a balanced one-way random-effects model

Source of Variation	Degrees of freedom	Mean square	Expected mean square
Factor A	$a-1$	$X_A/(a-1)$	$\sigma_e^2 + n\sigma_A^2$
Error	$an-a$	$X_E/(an-a)$	σ_e^2

Let $U_A = X_A/(\sigma_e^2 + n\sigma_A^2)$ and $U_E = X_E/\sigma_e^2$. Then U_A and U_E are independent chi-square distributed random variables with $a-1$ and $an-a$ degrees of freedom. Solving U_A and U_E for σ_A^2 and σ_e^2, and thereafter replacing the random variables X_A and X_E with their corresponding observed values x_A and x_E, respectively, the following GPQ for σ_A^2 is obtained:

$$G(\sigma_A^2) = \frac{x_A}{nU_A} - \frac{x_E}{nU_E}.$$

Regarding the two properties mentioned above; the distribution of $G(\sigma_A^2)$ is free of any unknown parameters. Moreover, at the observed sample value $G(\sigma_A^2)$ is free of nuisance parameters. Thus, $G(\sigma_A^2)$ is a GPQ for σ_A^2. The generalized confidence intervals for σ_A^2 can be obtained from the percentiles of the $G(\sigma_A^2)$ using Monte Carlo simulation.

3 Summary of the papers

3.1 Paper I

In this paper, we propose two modified variance component estimators, which are originally computed from the well known non-iterative variance component estimation method, Henderson's method 3. The estimators are expected to perform better than the original unbiased ones in terms of MSE.

A two-way linear mixed-effects model consisting of three variance components was considered. Two variance components, σ_1^2 and σ_2^2, are associated with the random effects in the model, and the third corresponds to the error variance component, σ_e^2. Our primary interest was σ_1^2. This variance component, denoted as σ_{u1}^2, was estimated as

$$\widehat{\sigma}_{u1}^2 = \frac{Y'AY}{a} - \frac{d(Y'BY)}{ab} + \frac{k(Y'CY)}{abc},$$

where A, B, C, a, b and c are defined as in Eq.(18) of Paper I and $k = \mathrm{tr}((P_{x_1} - P_x)V_2)\mathrm{tr}(P_{x_{12}} - P_{x_1}) - \mathrm{tr}(P_{x_1} - P_x)\mathrm{tr}((P_{x_{12}} - P_{x_1})V_2)$.

The mean square error of this unbiased variance component, denoted as $\mathrm{MSE}(\widehat{\sigma}_{u1}^2)$, was calculated as:

$$\begin{aligned}
\mathrm{MSE}(\widehat{\sigma}_{u1}^2) \;=\; & [\frac{2}{a^2}\mathrm{tr}(AV_1AV_1)]\sigma_1^4 \\
& + [\frac{2}{a^2}\mathrm{tr}(AV_2AV_2) + \frac{2d^2}{a^2b^2}\mathrm{tr}(BV_2BV_2)]\sigma_2^4 \\
& + [\frac{4}{a^2}\mathrm{tr}(AV_1AV_2)]\sigma_1^2\sigma_2^2 \\
& + [\frac{4}{a^2}\mathrm{tr}(A^2V_1)]\sigma_1^2\sigma_e^2 \\
& + [\frac{4}{a^2}\mathrm{tr}(AV_2A) + \frac{4d^2}{a^2b^2}\mathrm{tr}(BV_2B)]\sigma_2^2\sigma_e^2 \\
& + [\frac{2}{a^2}\mathrm{tr}(A^2) + \frac{2d^2}{a^2b^2}\mathrm{tr}(B^2) + \frac{2k^2}{a^2b^2c^2}\mathrm{tr}(C^2)]\sigma_e^4.
\end{aligned}$$

The variance component $\widehat{\sigma}^2_{u1}$ was modified by introducing perturbation coefficients into the standard unbiased estimator denoted by c_1, d_1 and d_2. The modified variance component estimator, denoted by $\widehat{\sigma}^2_{11}$ is:

$$\widehat{\sigma}^2_{11} = \frac{c_1}{a}(Y'AY - \frac{d}{b}d_1Y'BY + \frac{k}{bc}d_2Y'CY),$$

where A, B, C, a, b, c, d and k are all defined as previously for $\widehat{\sigma}^2_{u1}$. The mean square error of the modified estimator denoted by $\mathrm{MSE}(\widehat{\sigma}^2_{11})$ was calculated in Eq.(34) of Paper I. The values of c_1, d_1 and d_2 were chosen such that they minimize the coefficients in the leading terms of σ_1^4, σ_2^4 and σ_e^4 in $\mathrm{MSE}(\widehat{\sigma}^2_{11})$. Thereafter, the mean square errors of the modified and unmodified estimators were compared. In Proposition 1 and Theorem 1 of Paper I, the sufficient conditions under which $\mathrm{MSE}(\widehat{\sigma}^2_{11}) \leq \mathrm{MSE}(\widehat{\sigma}^2_{u1})$ holds are summarized.

The algorithm was intially published in the research report Al-Sarraj and von Rosen (2007), which also addressed the problem of non-uniqueness of the decomposition of the reduction in sums of squares. The number of variance components in a linear mixed-effects model consisting of three variance components $(\sigma_1^2, \sigma_2^2, \sigma_e^2)$, can be reduced from three to two variance components: σ_1^2 and σ_e^2, for instance by using an orthogonal projection. Thereafter, the variance component estimator $\widehat{\sigma}^2_1$ can be modified. To motivate the choice of the approach and which estimator to modify, two different decompositions of Henderson's method 3 were considered, referred to as Partition I and Partition II. Partition I is the decomposition and set of equations found in Paper I. In Partition II, a different set of reduction sums of squares are used, as specified in Section (4.2) of Al-Sarraj and von Rosen (2007), still with the focus on σ_1^2. The obtained estimators from both Partition I and Partition II were denoted by $\widehat{\sigma}^2_{u1}$ and $\widehat{\sigma}^2_1$, respectively. The obtained variance component estimator from Partition II was calculated as:

$$\widehat{\sigma}^2_1 = \frac{Y'(P_{x_{12}} - P_{x_2})Y}{\mathrm{tr}(P_{x_{12}} - P_{x_2})V_1} - \frac{\mathrm{tr}(P_{x_{12}} - P_{x_2})Y'(I - P_{x_{12}})Y}{\mathrm{tr}(P_{x_{12}} - P_{x_2})V_1\mathrm{tr}(I - P_{x_{12}})}. \qquad (27)$$

The variances of the two estimators obtained from the partitions I and II, denoted as $\mathrm{D}[\hat{\sigma}^2_{u1}]$ and $\mathrm{D}[\widehat{\sigma}^2_1]$, were compared. This comparison was substantiated by assuming the variance component estimator σ_2^2 to be diminutive to the extent rendering it acceptable to ignore its impact. Sufficient conditions under which $\mathrm{D}[\hat{\sigma}^2_{u1}] \leq \mathrm{D}[\hat{\sigma}^2_1]$ holds were summarized in Proposition 2 (Al-Sarraj and von Rosen, 2007). The chosen

partition, i.e. Partition I or Partition II, was based on the estimator with less variance. If the estimator from Partition I has less variance, the estimator is then modified as in Paper I, and if the estimator from Partition II has less variance, the modification suggested by Kelly and Mathew (1994) should be chosen.

3.2 Paper II

Variance component models are widely used in genetics, especially in animal breeding to study quantitative traits of individuals. In variance component QTL analysis, a mixed model is used to localize the most likely chromosome position of a QTL. In line with Paper I, the modified variance component estimator was applied on data from European wild boar $\times$ domestic pig intercross. For this, a mixed linear model consisting of three variance components was used to detect the most likely chromosome position of a QTL. A meat quality trait was examined. This trait is affected by the halothane gene located on chromosome 6 at position 80,4 cM. The model 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 estimators.

The REML and the two modified Henderson method 3 estimators of σ_1^2 were similar for most positions. The estimators at the halothane gene (80 cM) for REML, modified Partition I and modified Partition II, respectively, were: 4.96, 4.32, 5.06. A likelihood ratio (LR) was calculated at each position, as: $\text{LR} = -2(l_0 - l_1)$, where l_0 is the log-likelihood under the null hypothesis of no QTL (i.e. u_1 removed from the model) and l_1 is the log-likelihood for the full model at a specific position. The LR curve, obtained from fitting the variance component QTL model in the full model, using REML, showed a peak at 80 cM. 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.

3.3 Paper III

In this paper, the generalized prediction intervals (GPIs) for linear combinations of random effects in balanced and unbalanced random-effects models were derived, based on generalized inference methods of Tsui and Weerahandi (1989) and Weerahandi (1993). Six different models were considered; one- and two-way models, with and without

interaction. The random effects were assumed to be independently and normally distributed variables, with the GPIs for the quantities $\mu + u_1$, u_1 and $u_1 - u_2$ being of particular interest. Depending on the model, the quantities corresponded to a treatment mean, a treatment effect and a difference between two treatment effects, respectively.

Initially, the generalized pivotal quantities (GPQs) for the expected value and the variance components were constructed. The conditional distributions were computed for $(\mu + u_1|\bar{Y}_{1\cdot})$, $(u_1|\bar{Y}_{1\cdot} - \bar{Y}_{\cdot\cdot})$ and $(u_1 - u_2|\bar{Y}_{1\cdot} - \bar{Y}_{2\cdot})$, where $\bar{Y}_{1\cdot}$, $\bar{Y}_{1\cdot} - \bar{Y}_{\cdot\cdot}$ and $\bar{Y}_{1\cdot} - \bar{Y}_{2\cdot}$ represented averages in the considered models. The unknown parameters in the conditional distributions were replaced by their corresponding GPQs.

For each case of the different model layouts, 10 000 independent datasets were randomly generated. For each generated dataset, new independent samples of the GPQs were used. The $100(\alpha/2)$th and $100(1-\alpha/2)$th percentiles of the GPIs were obtained from the empirical conditional distributions and the estimated coverages were computed.

The simulation study was performed using the `lmer` function in `R` (Bates et al., 2015). The proposed GPIs were compared to corresponding REML-based prediction intervals of random effects (Pawitan, 2001):

$$\hat{u} \pm z_{\alpha/2} se(\hat{u}), \tag{28}$$

where $\hat{u}$ and $se(\hat{u})$ denote the estimated random effect and its estimated standard error, respectively. The $100(1-\alpha/2)$th percentiles of the standard normal distribution is denoted as $z_{\alpha/2}$. In (28) the approximate t-distributions can be used as an alternative, i.e. $\hat{u} \pm t_\nu se(\hat{u})$. The number of degrees of freedom ν can be approximated using the methods of Satterthwaite (1946) and Kenward and Roger (1997) alongside the containment method, which is the default method in the `mixed` procedure of the `SAS` software package. In almost all cases, estimated coverages using the GPI method were closer to the chosen nominal level than the ones obtained using the REML-method. The estimated coverages using the REML-based methods resulted in coverages lower than the nominal confidence level. This was also the case with the approximate methods of Satterthwaite (1946) and Kenward and Roger (1997).

3.4 Paper IV

This paper examined the accuracy of results in the software packages of the `mixed` procedure of `SAS` (Stroup et al., 2018) and the `lmer` function of `R` (Bates et al., 2015) when analyzing linear mixed-effects models

with the focus on randomized complete block experiments (RCB) and split-plot experiments. The implications were examined with regard to inference on fixed-effects, with focus on Type I errors.

The F-test statistics in the mixed-effects models are functions of the variance components associated with the random effects. In a simple analysis of variance setting, the variance components can be estimated by equating the mean square errors to their observed values, or by using likelihood-based methods, e.g. the REML method (Patterson and Thompson, 1971). The obtained estimates of the variance components can assume negative values. In some softwares, the estimates are constrained at zero, e.g. when applying the `lmer` function of `R` and the default of the `mixed` procedure in `SAS`. As such, the denominator degrees of freedom (ddf) of the F-test is implemented differently in `R` and in `SAS`, despite both using the Kenward and Roger (1997, 2009) method. However, with the `nobound` option of the `mixed` procedure, the variance component estimators are not constrained.

In addition, exact F-tests were calculated from three different types of sums of squares known as types I, II and III. In `R`, the parameter levels were constrained by setting the first level of the factor to zero (as implemented by `contr.treatment`), by setting the levels of the factors to sum up to zero (as implemented by `contr.sum`) and finally, by setting the last level of the factor to zero (as implemented by `contr.SAS`) (Fox and Weisberg, 2019).

The implications of the available options were examined in a randomized complete block experiment and in three split-plot experiments. The accuracy of results (F-test, ddf and p-value) in both software programmes were investigated alongside the exact F-test. Generally, with the default setting of the `mixed` procedure of `SAS` and the `lmer` function of `R`, the results differed considerably between the programmes. The degrees of freedom with the default setting of the `mixed` procedure of `SAS` was higher, resulting in more significant results.

Finally, 200 000 datasets were generated from a randomized complete block model and a split-plot model in a simulation study. The Type I error rates were estimated using six different layouts of each of the models. For the RCB model, the error variance was $\sigma_e^2 = 1$ and the block variance varied: $\sigma_B^2 = (0.1, 0.3, 0.5, 0.7, 0.9)$. In the split-plot model, the block and error variances were $\sigma_b^2 = 1$ and $\sigma_e^2 = 1$, respectively, while the main plot error variance varied: $\sigma_{AB}^2 = (0.1, 0.3, 0.5, 0.7, 0.9)$. Other terms in the model were assumed equal to zero. The analysis was performed using both `R` and `SAS`. In `R`, the

`lmer` and `Anova` functions were used. The type II test was used with the default parametrization `contr.treatment` and with the `contr.sum` parametrization. In the `mixed` procedure of `SAS`, both the default and the `nobound` options were investigated. The Kenward and Roger method, as implemented in the software, was applied to calculate the denominator degrees of freedom. In addition, using the `anova` procedure in `SAS`, the exact F-tests were computed with the denominator degrees of freedom of $(a-1)(b-1)$, where a is the number of treatment levels and b is the number of blocks in the experiment.

For the randomized complete block model, the simulation results in `R` showed too high frequency of Type I errors, especially when the block variance (σ_B^2) was low. Using the `nobound` option of the `mixed` procedure of `SAS`, the frequency of Type I errors was close to the nominal level 0.05, except for the layout with three treatment levels and two blocks, i.e. $\{a = 3, b = 2\}$. With the default option of the `mixed` procedure, the frequency of Type I errors was consistently larger than the nominal level.

For the split-plot model with a main plots, b blocks and c sub-plots, the following results were obtained. Using `R`, the frequency of Type I errors was below 0.05 when σ_{AB}^2 was small. With the `nobound` option of the `mixed` procedure of `SAS`, the frequency of Type I errors was close to the nominal level 0.05, except for two cases with the layouts $\{a = 2, b = 4, c = 12\}$ and $\{a = 3, b = 2, c = 12\}$ where the Type I error rates were too low. With the default setting of the `mixed` procedure of `SAS`, the frequency of Type I errors was almost always higher than the nominal level of 0.05.

4 General discussion

4.1 Research of this thesis

In analysis of experiments, considerable attention has been drawn to the importance of the variance component estimation in linear random- and mixed-effects models. When performing this type of analysis, there are certain issues a user may frequently face:

i. The choice of method of variance component estimation is ambiguous since there is no uniformly best method. Given that data are balanced, the results are identical regardless of the method used. If data are unbalanced, the outcomes may substantially differ, and this is almost always the case with real world data.

ii. Likelihood-based methods can in large data sets be computationally demanding. In addition, the methods do not provide any exact statistical inference except for the special case with balanced data.

iii. The methods used for variance component estimation may result in negative values of the variance estimates, despite the variance components being non-negative quantities.

The first two issues (i, ii) were addressed in Paper I and Paper II. The intention was to use a non-iterative variance component estimation method that is easy to compute and less time-consuming than the already well-established variance component estimation procedures such as the ML and REML procedures. The idea was to sacrifice unbiasedness, and instead obtain a smaller MSE in a certain class of estimators. The method used was based on Henderson's method 3. A linear mixed-effects model consisting of three variances was considered, where one of the variance components was of interest. This variance estimator

was improved such that the resulting estimator was expected to perform better in terms of MSE than the original estimator obtained from Henderson's method 3. In Paper II, the proposed estimator was tested on QTL data and compared with the likelihood-based procedures. The variances in the model were: 1) the variance between effects of base generation alleles, 2) the variance between effects of families, and 3) the residual variance. Of these, the first variance component mentioned was of interest.

The third issue (iii), the problem of negative variance estimates, is a major and frequently occurring problem, especially in small experiments or when many variance components are considered. Negative variance estimates are often set to zero. This practice leads to many inferential difficulties. For instance, prediction intervals for the random effects cannot be constructed using the traditional approximative methods of Satterthwaite (1946) and Kenward and Roger (1997, 2009). In Paper III, this problem was addressed where the generalized inference methodology, introduced by Tsui and Weerahandi (1989) and Weerahandi (1993), was utilized to derive and propose prediction intervals for random effects in some balanced and unbalanced model layouts.

When analyzing linear mixed-effects models in Paper IV, the availability of various software options posed a question as to which of the results obtained would offer a higher degree of accuracy. The `mixed` procedure of `SAS` (Stroup et al., 2018) and the `lmer` function of `R` (Bates et al., 2015) were examined in this paper. The implications for inference on the fixed effects were investigated with focus on Type I error.

4.2 Previous research

In the ANOVA-methodology, negative variance estimates are inevitable. How to deal with this issue has been presented by Searle (1971) and Searle et al. (1992). In practice, the most commonly used action in the case of a negative estimate is setting the value to zero. While other authors present alternative interpretations to negative variance estimates (Nelder, 1954; Smith and Murray, 1984; Stroup et al., 2018).

Instead of setting the negative ANOVA estimates directly to zero, Kelly and Mathew (1993, 1994) attempted to first improve the ANOVA-estimator in terms of MSE and then truncate the improved estimator at zero to obtain non-negative estimates. In terms of MSE, the truncated-at-zero improved estimator was considerably better

than the truncated ANOVA estimator. For a balanced one-way model, Kelly and Mathew (1993) improved the unbiased ANOVA variance estimator such that the resulting estimator had lower MSE and lower probability of negativity than the ANOVA-estimator. Kelly and Mathew (1994) used a similar approach and derived several non-negative estimators for mixed models with unbalanced data. The models they considered involved two variance components: one corresponding to the error variance, and the second being the parameter of interest. For models consisting of more than two variance components, they suggested using suitable linear transformations to reduce the number of variance components to two. Based on their simulation results on some unbalanced model layouts, the improved non-negative estimator performed better in terms of MSE than the non-negative estimator obtained from the ANOVA, ML and REML methods.

Henderson's method 3 is one of the three variance component estimation procedures proposed by Henderson (1953). The method can be used on linear mixed-effects models with or without interactions and with unbalanced data. This method relies crucially on the decomposition of the reduction sums of squares, which is not uniquely defined. In a two-way model without interaction consisting of three variance components (two for the random effects and one for the error component), two different decompositions of the Henderson's equations may be applied to estimate the variance component of interest, say σ_1^2. The two decompositions were referred to as Partition I and Partition II in Al-Sarraj and von Rosen (2007); the first consisting of all three variance components (σ_1^2, σ_2^2, σ_e^2) and the second consisting of two variance components (σ_1^2, σ_e^2). The variances obtained from Partition I can then be modified as in Paper I and the ones obtained from Partition II can be modified as in Kelly and Mathew (1994). The `R` code for both modified partitions is available on GitHub at `https://github.com/razaw1964/Modified-Henderson-method-3`.

Different layouts of a two-way model without interaction were considered in a simulation study conducted by Al-Sarraj et al. (2011). The performance of the variance component estimator obtained from the two partitions and their corresponding modified variance components were compared and evaluated. In addition, the comparison included variance component estimators obtained from the likelihood-based procedures, ML and REML. These six different estimators were compared in terms of MSE, squared bias and probability of obtaining negative estimates. The modified variance component estimator

obtained from Partition I was recommended over the one from Partition II when $\sigma_2^2/\sigma_1^2 < 1.0$. Furthermore, the MSEs of the modified Partition I estimator and the MLE estimator were very close and lower than all other estimators' MSEs.

Using the approximate methods of Satterthwaite (1946) and Kenward and Roger (1997, 2009), the prediction intervals for the random effects degenerate when a zero estimate occurs. To overcome this problem, Weerahandi (1993) used the generalized concept to obtain confidence intervals for cases where the classical approaches fail to provide reliable results. He introduced the generalized pivotal quantity (GPQ) which is used to construct confidence intervals; the intervals are referred to as generalized confidence intervals (GCIs). Several authors provided GCIs in many practical problems (Burdick et al., 2006; Chiang, 2001; Hannig et al., 2006; Iyer and Patterson, 2002; Krishnamoorthy et al., 2006; Wang et al., 2012; Weerahandi, 1995).

For a one-way random-effects model with balanced data, Gamage et al. (2013) derived the generalized prediction interval (GPI) for the predictor $\mu + k(\bar{y}_{1\cdot} - \mu)$, where μ denotes the overall mean, $\bar{y}_{1\cdot}$ the first treatment's mean and k the shrinkage factor: $k = n\sigma_A^2/(\sigma_e^2 + n\sigma_A^2)$.

When using the `mixed` procedure of `SAS` and the `lmer` function of `R` for the inference on the fixed effects, there is an opportunity to choose one of the three different sums of squares known as Type I, Type II and Type III. For balanced data layouts, all three types yield identical results, however, this is not the case for the unbalanced ones as the types correspond to different hypotheses being tested (Pendleton et al., 1986).

There has been considerable debate among authors as to which of the tests can be considered best; the Type III test was criticized by Nelder (1994) and Nelder and Lane (1995), explaining that a test of the main effects in the presence of an interaction was uninteresting, while others like Langsrud (2003) prefer the Type II test, as summarized by Maxwell et al. (2018). To date, there is no consensus in the literature as to which of these tests is best.

4.3 Main contributions

In this work, we have explored some options for explicit variance component estimation and introduced a new approach for prediction intervals of random effects. Moreover, some inference problems encountered when analyzing some simple models with two of the most commonly used statistical software packages, the `mixed` procedure of `SAS` and the

`lmer` function of `R`, were investigated.
The main contributions can be summarized as follows:

i. Two modified variance component estimators were proposed, each appropriate under certain given conditions. The estimators were easy to compute and had smaller MSE than the unmodified one (Paper I).

ii. The modified estimator applied on QTL data was easy to compute and less time-consuming than the ML and REML methods. The variance estimators were very close to the REML estimators at the QTL position with the likelihood ratio curve well approximated by the non-iterative variance component estimators (Paper II).

iii. The coverages of the proposed GPIs, derived for linear combinations of random effects, were closer to the chosen nominal level than the coverages of those obtained with REML-based methods (Paper III).

iv. With the default settings of the software packages `SAS` and `R`, the frequencies of Type I error differed greatly. This was attributed to the fact that the software packages computed the denominator degrees of freedom differently when the variance components were constrained at zero. The frequency of Type I error was close to the correct value when the `nobound` option of the `mixed` procedure of `SAS` was used. Inaccurate results were, however, occasionally obtained both with the `nobound` option of `SAS` and the default setting of `R` (Paper IV).

4.4 Suggestions for future work

Conducting a study to modify an estimator obtained from a model comprising four variance components would be of interest. Furthermore, finding necessary and sufficient conditions for the estimator to have a lower mean square error than the original unbiased estimator would pave the way for further research. The generalized prediction intervals in our studies were proposed for three treatment effects in six random-effects models with balanced and unbalanced data. A possible direction for future work may be to extend the idea to models also including fixed effects, i.e. mixed-effects models. Further research studies are particularly needed to investigate more general unbalanced linear

random- and mixed-effects models, and using the generalized method to compute intervals for a broader class of linear combinations of the random effects.

4.5 Conclusion

In this thesis we have modified an already existing non-iterative variance component estimation method, Henderson's method 3. Under 'easy to examine conditions' the proposed modified estimators have less mean square error. Applied to QTL data, the modified estimators demonstrated a good approximation to the REML estimates at the QTL position.

In our study, the generalized prediction intervals for some linear combinations of the random effects provided good results. The coverage probabilities for the proposed generalized prediction intervals outperformed the REML-based procedures and the approximate t-distribution methods of Satterthwaite (1946) and Kenward and Roger (1997).

As a final remark, standard procedures for analysis of linear mixed-effects models often provide approximate results when using statistical software. In cases where exact and explicit solutions exist, these should be preferred to the approximate methods.

While the present studies have introduced generalized prediction intervals for the random effects, and examined an alternative to the likelihood-based procedures for variance components estimation, there remains a need for further development of explicit non-iterative methods for analysis of linear mixed models.

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I

Improving Henderson's Method 3 Approach when Estimating Variance Components in a two-way mixed linear model

Razaw al Sarraj and Dietrich von Rosen

Abstract A two-way linear mixed model, consisting of three variance components, σ_1^2, σ_2^2 and σ_e^2 is considered. The variance component estimators are estimated using a well known non-iterative estimation procedure, Henderson's method 3. For σ_1^2 we propose two modified estimators. The modification is carried out by perturbing the standard estimator, such that the obtained estimator is expected to perform better in terms of its mean square error.

1 Introduction

In an analysis of variance context, the most commonly used method for estimating the variance components has been through equating the observed and expected mean squares, and solving a set of linear equations. As long as the data are balanced the ANOVA estimators are known to have good statistical properties, i.e., the obtained estimators are unbiased and have minimum variance among all unbiased estimators which are quadratic functions of the observations, see Graybill and Hultquist (1961). However, since real world data often are always unbalanced, this method is no longer appealing. For instance, the uniformly minimum variance property is lost. Furthermore, whether data are balanced or unbalanced, there is nothing in the ANOVA methodology that would prevent negative estimates of the variance components to occur, (LaMotte, 1973).

In a seminal paper Henderson (1953) considered variance component estimation with unbalanced data. He presented three methods of estimation which later on,

Razaw al Sarraj
Department of Energy and Technology, Box 7032, SE-750 07 Uppsala, Sweden,
e-mail: Razaw.Al-Sarraj@etsm.slu.se

Dietrich von Rosen
Department of Energy and Technology, Box 7032, SE-750 07 Uppsala, Sweden,
e-mail: dietrich.von.rosen@et.slu.se

came to be known as Henderson's method 1, 2 and 3. The obtained estimators are unbiased and translation invariant.

However, since all three methods are variations of the general ANOVA method, they suffer from the weaknesses of it. In particular, the lack of uniqueness.

In this paper we were motivated by Kelly and Mathew's (1994) work, where they improved the ANOVA estimators in a one-way variance component model. The model consists of two variance components, one is the random effect of interest, and the second is the error component. They modified the variance component estimator corresponding to the random effect such that the resulting estimator performed better than the unmodified ANOVA estimator in terms of the mean square error (MSE) criteria. If more components were to be included into the model, they were excluded by orthogonal projections. Hence, the model could always be dealt with as if it had two variance components.

Our aim is to modify the variance component estimators obtained by Henderson's method 3, in a two-way linear mixed model, i.e. a model with three variance components of which two components corresponding to the two random effects included in the model, and the third corresponds to the error component. Here, we want to emphasize that we are primarily interested in one of the variance components. We intend to modify this component and calculate its MSE. Thereafter, we compare it with the MSE of the unmodified one. This modified variance component estimator is expected to perform better in terms of the MSE criteria.

1.1 Quadratic forms

Estimation of variance components for balanced and unbalanced data are based on quadratic forms $Y'AY$ where A is a symmetric matrix, and

$$Y \sim N(\mu, V).$$

In particular the mean and the variance of $Y'AY$ are needed.

(i) The mean of $Y'AY$, is equal to

$$\mathrm{E}(Y'AY) = \mathrm{tr}(AV) + \mu'A\mu, \tag{1}$$

which is true even if Y is not normally distributed.

(ii)the variance of $Y'AY$ is

$$\mathrm{D}[Y'AY] = 2\mathrm{tr}(AVAV) + 4(\mu'AVA\mu). \tag{2}$$

(iii)If AV is idempotent, the distribution of $Y'AY$ is given by

$$Y'AY \sim \chi^2(r_A, \frac{1}{2}\mu'A\mu),$$

where $\chi^2(r_A, \frac{1}{2}\mu' A\mu)$ is non-central chi-square distribution, with degrees of freedom equal to r_A, i.e., the rank of A, and the non-centrality parameter $\frac{1}{2}\mu' A\mu$.

1.2 Important criteria for deriving estimators

Consider the following mixed linear model

$$Y = X\beta + Zu + e, \tag{3}$$

where Y is the $N \times 1$ vector of observations, X is a known $N \times m$ matrix, β is an $m \times 1$ vector of unknown fixed effect parameters, and e is an $N \times 1$ vector of random error with mean 0 and dispersion matrix $\sigma_e^2 I_N$. The term Zu given in model (3) is a random term that can be partitioned conformably as

$$Zu = \begin{bmatrix} Z_1 & Z_2 & \dots & Z_r \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \\ \vdots \\ u_r \end{bmatrix} = \sum_{i=1}^{r} Z_i u_i.$$

Thus, model (3) can be rewritten as

$$Y = X\beta + \sum_{i=1}^{r} Z_i u_i + e, \tag{4}$$

where Z_i is $N \times n_i$ incidence matrix of known elements, u_i is $n_i \times 1$ vector of random effects, with zero mean value and dispersion matrix $\sigma_i^2 I_{n_i}$, $i = 1, \cdots, r$. Further it is assumed that the u_i and e are uncorrelated random variables. Then from (4), $\mathrm{E}(Y) = X\beta$ and the dispersion matrix $\mathrm{V} = D[Y] = \Sigma_{i=1}^{r} Z_i Z_i' \sigma_i^2 + \sigma_e^2 I_N$. The parameters σ_i^2 and σ_e^2 are unknown. Since Zu and e are random effects, they can be combined into one random term. Thus (4) can be rewritten as $Y = X\beta + \Sigma_{i=0}^{r} Z_i u_i$ and the dispersion matrix $\mathrm{V} = \Sigma_{i=0}^{r} Z_i Z_i' \sigma_i^2$, where $u_0 = e$, $\sigma_0^2 = \sigma_e^2$ and $Z_0 = I_N$.
To generalize the idea of estimating a single variance component, we consider estimating a linear function of the variance components, $p_0\sigma_0^2 + p_1\sigma_2^2 + \cdots + p_r\sigma_r^2$, where p_i are known, by a quadratic function $Y'AY$ of the random variable Y in (4). The matrix A should be chosen according to some suitable criteria.

(i) Unbiasedness: If $Y'AY$ is unbiased for $\sum_{i=0}^{r} p_i\sigma_i^2$ for all σ_i^2, then under the restriction $X'AX = 0$,

$$\mathrm{E}(Y'AY) = \mathrm{tr}(AV) = \sum_{i=0}^{r} \mathrm{tr}(AZ_i Z_i')\sigma_i^2 = \sum_{i=0}^{r} p_i\sigma_i^2. \tag{5}$$

i.e., an unbiased estimator is obtained if $p_i = \mathrm{tr}(AZ_i Z_i')$.

(ii) Translation Invariance: $Y'AY$ is translation invariant if it's value is not affected by any change in the fixed effect parameter for the model. If instead of β we consider $\gamma = \beta - \beta_0$ as the unknown parameter, where β_0 is fixed. Then $Y'AY$ is translation invariant if $Y'AY = (Y - X\gamma)'A(Y - X\gamma)$ for all γ. Thus $AX = 0$. Since $AX = 0$ always implies $X'AX = 0$, we also have the unbiasedness condition satisfied. However, the reverse is not true i.e., unbiasedness does not imply invariance except when A is n.n.d..

(iii) Minimum Variance: The variance of $Y'AY$ under a normality assumption equals

$$\mathrm{D}[Y'AY] = 2\mathrm{tr}[AVAV] + 4\beta'X'AVAX\beta. \tag{6}$$

Under unbiasedness i.e., $AX = 0$, the variance reduces to

$$\mathrm{D}[Y'AY] = 2\mathrm{tr}[AVAV].$$

The mean squared error, of $Y'AY$ equals

$$\mathrm{MSE}[Y'AY] = \mathrm{D}[Y'AY] + [\mathrm{Bias}(Y'AY)]^2. \tag{7}$$

Using the condition for translation invariance $AX = 0$ and unbiasedness $\mathrm{tr}[AZ_iZ_i'] = p_i$, equation (7) reduces to

$$\mathrm{MSE}[Y'AY] = D[Y'AY] = 2\mathrm{tr}[AVAV].$$

Both (6) and (7), under unbiasedness and invariance reduce to $2\mathrm{tr}(AVAV)$.

1.3 ANOVA- based methods of estimation

This method is derived by equating the sums of squares in an analysis of variance table to their expected values. Let σ^2 be the vector of variance components to be estimated in some model, and let s be a vector of sums of squares. Then taking the expected value

$$E(s) = C\sigma^2, \tag{8}$$

where C is a non-singular matrix, the ANOVA estimator of $\widehat{\sigma}^2$ is based on (8) and is the solution to $s = C\widehat{\sigma}^2$, which equals

$$\widehat{\sigma}^2 = C^{-1}s. \tag{9}$$

The expression in (8) can be extended to include not only sums of squares but also any set of quadratic forms. Let $q = (q_1, q_2, \cdots, q_m)'$ be the $m \times 1$ vector of quadratic forms such that

$$E(q) = A\sigma^2, \tag{10}$$

where $\sigma^2 = (\sigma_1^2, \sigma_2^2, \ldots, \sigma_k^2)'$ is the vector of $k \times 1$ variance components and A being an $m \times k$ matrix of known coefficients. Then, if $m = k$ and A is non-singular, (10) will give $\widehat{\sigma}^2 = A^{-1}q$ as an unbiased estimator of σ^2, as in (9). In cases when there are more quadratic forms than there are variance components to estimate, the following formula gives an unbiased estimator: $\widehat{\sigma}^2 = (A'A)^{-1}A'q$, (see Searle, Casella & McCulloch 1992).

2 Henderson's three methods

Henderson (1953) presented in his paper three methods of estimation of variance components, currently known as Henderson's method 1, 2 and 3. This paper is considered to be the landmark work of dealing with the problem of estimation of variance components for unbalanced data. For balanced data, variances are usually estimated using the minimum variance estimators based on the sums of squares, appearing in the analysis of variance table. For unbalanced data the situation is different; it is not always clear which mean squares should be used (see Searle 1971). Henderson's methods are sometimes described as being three different ways of using the general ANOVA-method (Searle 1987). They differ only in the different quadratics (not always sums of squares), used for a vector of any linearly independent quadratic forms of observations. All three methods involve calculations of mean squares, taking their expected values, equating them to the observed ones, and then solving the resulting equations in order to obtain the variance component estimators. Some of the merits of the methods is that they are easy to compute, they require no strong distributional assumptions, and by construction these methods yield unbiased estimators. However, the estimators can fall outside the parameter space, i.e., they can become negative. Moreover, the estimators are not unique, because when there are several random effects, the sums of squares for them can be computed in several ways, i.e, corrected for several combinations of other effects. When data are balanced, all three methods reduce to the usual ANOVA-method. For a review of all three methods, see Searle (1968). In our work, we will be concentrating on Henderson's method 3.

2.1 Method 3

This method can be used on mixed models with or without interactions. Instead of the sums of squares that method 1 and 2 use, method 3 uses reductions in sums of squares due to fitting sub-models of the full model, and then equating the reduced sums of squares to their respective expected values. The outcome will be a set of linear estimation equations, which have to be solved in order to obtain the variance component estimators. The drawback with this method is that sometimes

more reduction sum of squares are available than necessary to estimate the variance component estimators (see Searle 1987). In other words, occasionally more than one set of estimating equations for the variance components can be computed for one model. From each set we get different estimators of the variance components. Which set of estimators to prefer is not clear, i.e., the variance component estimators are not unique. We will consider the following two-way mixed model with no interaction,

$$Y = X\beta + Z_1 u_1 + Z_2 u_2 + e, \quad \text{(full model)} \tag{11}$$

where β is the fixed parameter vector and u_1, u_2 are random effect parameters. For this model there are three variance components to estimate, i.e., the variance of the two random effects denoted by σ_1^2 and σ_2^2 respectively, and the third is the error variance component denoted by σ_e^2. We may obtain several sets of estimation equations. The sub-models which may give estimation equations are,

$$Y = X\beta + e, \tag{12}$$

$$Y = X\beta + Z_1 u_1 + e, \tag{13}$$

$$Y = X\beta + Z_2 u_2 + e. \tag{14}$$

Now we present some special notation for reduction sum of squares which was used by Searle (1971, 1987). Let R(.) denote the reduction sum of squares. The sum of squares used for estimation corresponding to the sub-models (12), (13) and (14) can according to this notation be expressed as, $\mathrm{R}(\beta)$, $\mathrm{R}(\beta, u_1)$ and $\mathrm{R}(\beta, u_2)$, respectively. Another notation which will be needed before we write the possible set of equations is R(./.) which is the reduction sum of squares due to fitting the full model (11) minus that of the sub-model. For (11) two sets of estimation equations may be considered

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

where SSE denotes the residual sum of squares. For the first set of estimation equations we define the following partitioned matrices: $[X]$, $[X, Z_1]$ and $[X, Z_1, Z_2]$. Each reduction R(./.) can be expressed in the form $Y'AY$ for some symmetric matrix A. Define the projection matrix P_w=$w(w'w)^- w'$. Thus P_w is an idempotent matrix, for more properties see Schott (1997). Assuming normality all the reduction sum of squares follow a non-central χ^2 distribution and all these reduction sum of squares are independent of each other and of SSE, see Searle (1987). We shall be using the first set of estimation equation in the first part of the work. In the second part, i.e., in section 4, different reductions in sums of squares will be compared. For the first set of equations we need to define the following projection matrices,

$$P_x = X(X'X)^- X', \tag{15}$$

$$P_{x1} = (X,Z_1)((X,Z_1)'(X,Z_1))^{-}(X,Z_1)', \tag{16}$$

$$P_{x_{12}} = (X,Z_1,Z_2)((X,Z_1,Z_2)'(X,Z_1,Z_2))^{-}(X,Z_1,Z_2)'. \tag{17}$$

The reduction sums of squares R(./.) can now be obtained as.

$$\begin{aligned} \mathrm{R}(u_1/\beta) &= \mathrm{R}(u_1,\beta) - \mathrm{R}(\beta) \\ &= Y'(P_{x_1} - P_x)Y, \end{aligned}$$

$$\begin{aligned} \mathrm{R}(u_2/\beta,u_1) &= \mathrm{R}(\beta,u_1,u_2) - \mathrm{R}(\beta,u_1) \\ &= Y'(P_{x_{12}} - P_{x_1})Y, \end{aligned}$$

and

$$\mathrm{SSE} = Y'(I - P_{x_{12}})Y.$$

To apply the procedure, the expected values of the reduction sums of squares are computed. Thereafter the expected values are to be equated to their observed values and by solving the obtained equations the variance components are obtained. The expression for the expected value given in (1), can be used since the dispersion matrix, denoted by V is $V{=}\sigma_1^2V_1{+}\sigma_2^2V_2{+}\sigma_e^2I$, where $V_1{=}Z_1Z_1'$ and $V_2{=}Z_2Z_2'$. The following is obtained

$$\mathrm{E}[\mathrm{R}(u_1/\beta)] = \mathrm{tr}(P_{x_1} - P_x)[\sigma_1^2V_1 + \sigma_2^2V_2 + \sigma_e^2I],$$

$$\mathrm{ER}(u_2/\beta,u_1) = \mathrm{tr}(P_{x_{12}} - P_{x_1})[\sigma_1^2V_1 + \sigma_2^2V_2 + \sigma_e^2I],$$

and

$$\mathrm{E}[\mathrm{SSE}] = \mathrm{tr}(I - P_{x_{12}})[\sigma_1^2V_1 + \sigma_2^2V_2 + \sigma_e^2I].$$

The set of calculated reduction sum of squares may be arranged in a vector. Thereafter by equating these expected values to the observed ones we get

$$\begin{bmatrix} Y'(P_{x_1} - P_x)Y \\ Y'(P_{x_{12}} - P_{x_1})Y \\ Y'(I - P_{x_{12}})Y \end{bmatrix} = J \begin{bmatrix} \sigma_1^2 \\ \sigma_2^2 \\ \sigma_e^2 \end{bmatrix},$$

where

$$J = \begin{bmatrix} \mathrm{tr}(P_{x_1} - P_x)V_1 & \mathrm{tr}(P_{x_1} - P_x)V_2 & \mathrm{tr}(P_{x_1} - P_x)I \\ \mathrm{tr}(P_{x_{12}} - P_{x_1})V_1 & \mathrm{tr}(P_{x_{12}} - P_{x_1})V_2 & \mathrm{tr}(P_{x_{12}} - P_{x_1})I \\ \mathrm{tr}(I - P_{x_{12}})V_1 & \mathrm{tr}(I - P_{x_{12}})V_2 & \mathrm{tr}(I - P_{x_{12}})I \end{bmatrix}.$$

Thus, the estimators of the variance components are

$$\begin{bmatrix} \widehat{\sigma}_1^2 \\ \widehat{\sigma}_2^2 \\ \widehat{\sigma}_e^2 \end{bmatrix} = J^{-1} \begin{bmatrix} Y'(P_{x_1} - P_x)Y \\ Y'(P_{x_{12}} - P_{x_1})Y \\ Y'(I - P_{x_{12}})Y \end{bmatrix}.$$

However, since $P_{x_1}V_1 = V_1$, $P_{x_{12}}V_2 = V_2$ and $P_{x_{12}}V_1 = V_1$, the J matrix reduces to

$$J = \begin{bmatrix} \mathrm{tr}(P_{x_1} - P_x)V_1 & \mathrm{tr}(P_{x_1} - P_x)V_2 & \mathrm{tr}(P_{x_1} - P_x) \\ 0 & \mathrm{tr}(P_{x_{12}} - P_{x_1})V_2 & \mathrm{tr}(P_{x_{12}} - P_{x_1}) \\ 0 & 0 & \mathrm{tr}(I - P_{x_{12}}) \end{bmatrix}.$$

Let

$$\begin{aligned} &A = (P_{x_1} - P_x), \quad B = (P_{x_{12}} - P_{x_1}), \quad C = (I - P_{x_{12}}), \\ &a = tr(P_{x_1} - P_x)V_1, \quad b = tr(P_{x_{12}} - P_{x_1})V_2, \quad c = tr(I - P_{x12}), \\ &d = tr(P_{x_1} - P_x)V_2, \quad e = tr(P_{x_{12}} - P_{x_1}), \quad f = tr(P_{x_1} - P_x)), \end{aligned} \tag{18}$$

we note that A, B and C are idempotent matrices. Using these notations the estimation equations can be written as

$$\begin{bmatrix} \widehat{\sigma}_1^2 \\ \widehat{\sigma}_2^2 \\ \widehat{\sigma}_e^2 \end{bmatrix} = J^{-1} \begin{bmatrix} Y'AY \\ Y'BY \\ Y'CY \end{bmatrix}, \tag{19}$$

The variance component estimator of σ_1^2, denoted by $\widehat{\sigma}_{u1}^2$ is:

$$\begin{aligned} \widehat{\sigma}_{u1}^2 \; = & \frac{\mathrm{tr}((P_{x_{12}} - P_{x_1})V_2)\mathrm{tr}(I - P_{x_{12}})Y'(P_{x_1} - P_x)Y}{\mathrm{tr}((P_{x_1} - P_x)V_1)\mathrm{tr}((P_{x_{12}} - P_{x_1})V_2)\mathrm{tr}(I - P_{x_{12}})} \\ & - \frac{\mathrm{tr}((P_{x_1} - P_x)V_2)\mathrm{tr}(I - P_{x_{12}})Y'(P_{x_{12}} - P_{x_1})Y}{\mathrm{tr}((P_{x_1} - P_x)V_1)\mathrm{tr}((P_{x_{12}} - P_{x_1})V_2)\mathrm{tr}(I - P_{x_{12}})} \\ & + \frac{kY'(I - P_{x_{12}})Y}{\mathrm{tr}((P_{x_1} - P_x)V_1)\mathrm{tr}((P_{x_{12}} - P_{x_1})V_2)\mathrm{tr}(I - P_{x_{12}})}, \end{aligned} \tag{20}$$

where $k = \mathrm{tr}((P_{x_1} - P_x)V_2)\mathrm{tr}(P_{x_{12}} - P_{x_1}) - \mathrm{tr}(P_{x_1} - P_x)\mathrm{tr}((P_{x_{12}} - P_{x_1})V_2)$. Equation (20) simplifies to

$$\begin{aligned} \widehat{\sigma}_{u1}^2 \; = & \frac{Y'(P_{x_1} - P_x)Y}{\mathrm{tr}((P_{x_1} - P_x)V_1)} - \frac{\mathrm{tr}((P_{x_1} - P_x)V_2)Y'(P_{x_{12}} - P_{x_1})Y}{\mathrm{tr}((P_{x_1} - P_x)V_1)\mathrm{tr}((P_{x_{12}} - P_{x_1})V_2)} \\ & + \frac{kY'(I - P_{x_{12}})Y}{\mathrm{tr}((P_{x_1} - P_x)V_1)\mathrm{tr}((P_{x_{12}} - P_{x_1})V_2)\mathrm{tr}(I - P_{x_{12}})}. \end{aligned} \tag{21}$$

Using the previous notations we can write $\widehat{\sigma}_{u1}^2$ as

$$\widehat{\sigma}_{u1}^2 = \frac{Y'AY}{a} - \frac{d(Y'BY)}{ab} + \frac{k(Y'CY)}{abc}, \tag{22}$$

where A, B, C, b, c and e are defined as in (18). Despite the fact that in our study we will focus on one of the variance components we also give the estimators of the two other components which may be calculated from (19);

$$\widehat{\sigma}^2_{u_2} = \frac{\mathrm{tr}(I-P_{x_{12}})Y'(P_{x_{12}}-P_{x_1})Y}{\mathrm{tr}((P_{x_{12}}-P_{x_1})V_2)\mathrm{tr}(I-P_{x_{12}})} - \frac{\mathrm{tr}(P_{x_{12}}-P_{x_1})Y'(I-P_{x_{12}})Y}{\mathrm{tr}((P_{x_{12}}-P_{x_1})V_2)\mathrm{tr}(I-P_{x_{12}})},$$

$$\widehat{\sigma}^2_e = \frac{\mathrm{tr}((P_{x_1}-P_x)V_1)\mathrm{tr}((P_{x_{12}}-P_{x_1})V_2)Y'(I-P_{x_{12}})Y}{\mathrm{tr}((P_{x_1}-P_x)V_1)\mathrm{tr}((P_{x_{12}}-P_{x_1})V_2)\mathrm{tr}(I-P_{x_2})} = \frac{Y'(I-P_{x_{12}})Y}{\mathrm{tr}(I-P_{x_{12}})}.$$

2.1.1 Mean Square Error of $\widehat{\sigma}^2_{u1}$

The mean square of $\widehat{\sigma}^2_{u1}$ equals its variance since $\widehat{\sigma}^2_{u1}$ is an unbiased estimator,

$$\begin{aligned} MSE(\widehat{\sigma}^2_{u1}) &= D[\widehat{\sigma}^2_{u1}] = D\left[\frac{Y'AY}{a} - \frac{d(Y'BY)}{ab} + \frac{k(Y'CY)}{abc}\right] \\ &= \frac{1}{a^2}D[Y'AY] + \frac{d^2}{a^2b^2}D[Y'BY] + \frac{k^2}{a^2b^2c^2}D[Y'CY] \\ &= \frac{2}{a}\mathrm{tr}[AV]^2 + \frac{2d^2}{a^2b^2}\mathrm{tr}[BV]^2 + \frac{2k^2}{a^2b^2c^2}\mathrm{tr}[CV]^2, \end{aligned} \tag{23}$$

Moreover since all the involved quadratic forms are uncorrelated, $V = \sigma^2_1 V_1 + \sigma^2_2 V_2 + \sigma^2_e I$ and the MSE equals

$$\mathrm{D}[\widehat{\sigma}^2_{u_1}] = A_1 + A_2 + A_3, \tag{24}$$

where

$$\begin{aligned} A_1 = \frac{2}{a^2}[&\mathrm{tr}(AV_1AV_1)\sigma^4_1 + 2\mathrm{tr}(AV_1AV_2)\sigma^2_1\sigma^2_2 + \mathrm{tr}(AV_2AV_2)\sigma^4_2 + 2\mathrm{tr}(AV_1A)\sigma^2_1\sigma^2_e \\ &+ 2\mathrm{tr}(AV_2A)\sigma^2_2\sigma^2_e + \mathrm{tr}(A^2)\sigma^4_e], \end{aligned}$$

$$\begin{aligned} A_2 = \frac{2d^2}{a^2b^2}[&\mathrm{tr}(BV_1BV_1)\sigma^4_1 + 2\mathrm{tr}(BV_1BV_2)\sigma^2_1\sigma^2_2 + \mathrm{tr}(BV_2BV_2)\sigma^4_2 + 2\mathrm{tr}(BV_1B)\sigma^2_1\sigma^2_e \\ &+ 2\mathrm{tr}(BV_2B)\sigma^2_2\sigma^2_e + \mathrm{tr}(B^2)\sigma^4_e], \end{aligned}$$

$$\begin{aligned} A_3 = \frac{2k^2}{a^2b^2c^2}[&\mathrm{tr}(CV_1CV_1)\sigma^4_1 + 2\mathrm{tr}(CV_1CV_2)\sigma^2_1\sigma^2_2 + \mathrm{tr}(CV_2CV_2)\sigma^4_2 \\ &+ 2\mathrm{tr}(CV_1C)\sigma^2_1\sigma^2_e + 2\mathrm{tr}(CV_2C)\sigma^2_2\sigma^2_e + \mathrm{tr}(C^2)\sigma^4_e]. \end{aligned}$$

Thus, the following MSE is obtained:

$$
\begin{aligned}
\mathrm{MSE}(\widehat{\sigma}^2_{u1}) &= \left[\frac{2}{a^2}\mathrm{tr}(AV_1AV_1)+\frac{2d^2}{a^2b^2}\mathrm{tr}(BV_1BV_1)+\frac{2k^2}{a^2b^2c^2}\mathrm{tr}(CV_1CV_1)\right]\sigma_1^4\\
&+\left[\frac{2}{a^2}\mathrm{tr}(AV_2AV_2)+\frac{2d^2}{a^2b^2}\mathrm{tr}(BV_2BV_2)+\frac{2k^2}{a^2b^2c^2}\mathrm{tr}(CV_2CV_2)\right]\sigma_2^4\\
&+\left[\frac{4}{a^2}\mathrm{tr}(AV_1AV_2)+\frac{4d^2}{a^2b^2}\mathrm{tr}(BV_1BV_2)+\frac{4k^2}{a^2b^2c^2}\mathrm{tr}(CV_1CV_2)\right]\sigma_1^2\sigma_2^2\\
&+\left[\frac{4}{a^2}\mathrm{tr}(A^2V_1)+\frac{4d^2}{a^2b^2}\mathrm{tr}(B^2V_1)+\frac{4k^2}{a^2b^2c^2}\mathrm{tr}(C^2V_1)\right]\sigma_1^2\sigma_e^2\\
&+\left[\frac{4}{a^2}\mathrm{tr}(A^2V_2)+\frac{4d^2}{a^2b^2}\mathrm{tr}(B^2V_2)+\frac{4k^2}{a^2b^2c^2}\mathrm{tr}(C^2V_2)\right]\sigma_2^2\sigma_e^2\\
&+\left[\frac{2}{a^2}\mathrm{tr}(A^2)+\frac{2d^2}{a^2b^2}\mathrm{tr}(B^2)+\frac{2k^2}{a^2b^2c^2}\mathrm{tr}(C^2)\right]\sigma_e^4,
\end{aligned}
$$

since $\mathrm{tr}(CV_1)=0$, $\mathrm{tr}(CV_2)=0$ and $\mathrm{tr}(BV_1)=0$. The above can be simplified to

$$
\begin{aligned}
\mathrm{MSE}(\widehat{\sigma}^2_{u1}) = &\left[\frac{2}{a^2}\mathrm{tr}(AV_1AV_1)\right]\sigma_1^4+\left[\frac{2}{a^2}\mathrm{tr}(AV_2AV_2)+\frac{2d^2}{a^2b^2}\mathrm{tr}(BV_2BV_2)\right]\sigma_2^4\\
&+\left[\frac{4}{a^2}\mathrm{tr}(AV_1AV_2)\right]\sigma_1^2\sigma_2^2+\left[\frac{4}{a^2}\mathrm{tr}(A^2V_1)\right]\sigma_1^2\sigma_e^2\\
&+\left[\frac{4}{a^2}\mathrm{tr}(AV_2A)+\frac{4d^2}{a^2b^2}\mathrm{tr}(BV_2B)\right]\sigma_2^2\sigma_e^2\\
&+\left[\frac{2}{a^2}\mathrm{tr}(A^2)+\frac{2d^2}{a^2b^2}\mathrm{tr}(B^2)+\frac{2k^2}{a^2b^2c^2}\mathrm{tr}(C^2)\right]\sigma_e^4. \qquad (25)
\end{aligned}
$$

3 Perturbing Henderson's equation

In this section, we modify the variance component estimators obtained by Henderson's method 3. This modification is carried out by perturbing the Henderson's estimation equation. Thus, the obtained variance component estimators are biased. Thereafter, by using some suitable criterion, for instance, the MSE, we evaluate the performance of the estimator by comparing it with the MSE of the unmodified estimator. For the estimation equation (19), we define a new class of estimators

$$\begin{bmatrix} c_1 Y'AY \\ c_1 d_1 Y'BY \\ c_1 d_2 Y'CY \end{bmatrix} = J \begin{bmatrix} \sigma_1^2 \\ \sigma_2^2 \\ \sigma_e^2 \end{bmatrix} \tag{26}$$

where J is defined in section (2.1), and $c_1 \geq 0$, d_1 and d_2 are constants to be determined such that it would minimize the leading terms in the MSE of the estimator. The resulting estimator will perform better in terms of MSE since $c_1 = d_1 = d_2 = 1$ gives the same MSE. Thus, the modified variance component estimator of σ_1^2, denoted by $\widehat{\sigma}_{11}^2$ is

$$\widehat{\sigma}_{11}^2 = \frac{c_1}{a}\left(Y'AY - \frac{d}{b}d_1 Y'BY + \frac{k}{bc}d_2 Y'CY\right), \tag{27}$$

where A, B, C, a, b, c and d are all defined in (18). The MSE of this modified variance component is

$$\mathrm{MSE}[\widehat{\sigma}_{11}^2] = \mathrm{D}[\widehat{\sigma}_{11}^2] + [\mathrm{E}(\widehat{\sigma}_{11}^2) - \sigma_1^2]^2. \tag{28}$$

Since now (19) is perturbed, the estimator is not unbiased, The variance in (27) equals

$$\mathrm{D}[\widehat{\sigma}_{11}^2] = \frac{c_1^2}{a^2}\mathrm{D}[Y'AY] + \frac{d^2 c_1^2 d_1^2}{a^2 b^2}\mathrm{D}[Y'BY] + \frac{k^2 c_1^2 d_2^2}{a^2 b^2 c^2} D[Y'CY],$$

since $\mathrm{D}[\widehat{\sigma}_{11}^2]$ has the same structure as (25). Hence the variance of the modified estimator $\widehat{\sigma}_{11}^2$ can be written

$$\begin{aligned} \mathrm{D}[\widehat{\sigma}_{11}^2] = {} & \left[\frac{2c_1^2}{a^2}\mathrm{tr}(AV_1AV_1)\right]\sigma_1^4 + \left[\frac{2c_1^2}{a^2}tr(AV_2AV_2) + \frac{2d^2c_1^2d_1^2}{a^2b^2}tr(BV_2BV_2)\right]\sigma_2^4 \\ & + \left[\frac{4c_1^2}{a^2}tr(AV_1AV_2)\right]\sigma_1^2\sigma_2^2 \\ & + \left[\frac{4c_1^2}{a^2}tr(A^2V_1)\right]\sigma_1^2\sigma_e^2 + \left[\frac{4c_1^2}{a^2}tr(AV_2A) + \frac{4d^2c_1^2d_1^2}{a^2b^2}tr(BV_2B)\right]\sigma_2^2\sigma_e^2 \\ & + \left[\frac{2c_1^2}{a^2}tr(A^2) + \frac{2d^2c_1^2d_1^2}{a^2b^2}tr(B^2) + \frac{2k^2c_1^2d_2^2}{a^2b^2c^2}tr(C^2)\right]\sigma_e^4. \end{aligned} \tag{29}$$

Now we will calculate the bias part of (27), and thus the expectation of $\widehat{\sigma}_{11}^2$ is needed:

$$\begin{aligned}E[\widehat{\sigma}_{11}^2] &= \frac{c_1}{a}E(Y'AY) - \frac{dc_1}{ab}d_1E(Y'BY) + \frac{c_1kd_2}{abc}E(Y'CY)\\ &= \frac{c_1}{a}tr[A(\sigma_1^2V_1 + \sigma_2^2V_2 + \sigma_e^2I)]\\ &- \frac{dc_1d_1}{ab}tr[B(\sigma_1^2V_1 + \sigma_2^2V_2 + \sigma_e^2I)]\\ &+ \frac{c_1kd_2}{abc}tr[C(\sigma_1^2V_1 + \sigma_2^2V_2 + \sigma_e^2I)],\end{aligned}$$

which can be simplified to

$$\begin{aligned}E[\widehat{\sigma}_{11}^2] &= \left[\frac{c_1}{a}tr(AV_1) - \frac{dc_1d_1}{ab}tr(BV_1) + \frac{c_1kd_2}{abc}tr(CV_1)\right]\sigma_1^2\\ &+ \left[\frac{c_1}{a}tr(AV_2) - \frac{dc_1d_1}{ab}tr(BV_2) + \frac{c_1kd_2}{abc}tr(CV_2)\right]\sigma_2^2\\ &+ \left[\frac{c_1}{a}tr(A) - \frac{dc_1d_1}{ab}tr(B) + \frac{c_1kd_2}{abc}tr(C)\right]\sigma_e^2. \qquad (30)\end{aligned}$$

Thus, the squared bias can be written

$$\begin{aligned}(E[\widehat{\sigma}_{11}^2] - \sigma_1^2)^2 = &\left[\left(\frac{c_1}{a}tr(AV_1) - 1\right)\sigma_1^2 + \left(\frac{c_1}{a}tr(AV_2) - \frac{dc_1d_1}{ab}tr(BV_2)\right)\sigma_2^2\right.\\ &\left. + \left(\frac{c_1}{a}tr(A) - \frac{dc_1d_1}{ab}tr(B) + \frac{c_1kd_2}{abc}tr(C)\right)\sigma_e^2\right]^2. \qquad (31)\end{aligned}$$

If we substitute the variance and biased part back into (28), we get the following:

$$\begin{aligned}\mathrm{MSE}(\widehat{\sigma}_{11}^2) = &\left[\frac{2c_1^2}{a^2}tr(AV_1AV_1)\right]\sigma_1^4 + \left[\frac{4c_1^2}{a^2}tr(AV_1AV_2)\right]\sigma_1^2\sigma_2^2\\ &+ \left[\frac{2c_1^2}{a^2}tr(AV_2AV_2) + \frac{2d^2c_1^2d_1^2}{a^2b^2}tr(BV_2BV_2)\right]\sigma_2^4\\ &+ \left[\frac{4c_1^2}{a^2}tr(A^2V_1)\right]\sigma_1^2\sigma_e^2\\ &+ \left[\frac{4c_1^2}{a^2}tr(A^2V_2) + \frac{4d^2c_1^2d_1^2}{a^2b^2}tr(B^2V_2)\right]\sigma_2^2\sigma_e^2\\ &+ \left[\frac{2c_1^2}{a^2}tr(A^2) + \frac{2d^2c_1^2d_1^2}{a^2b^2}tr(B^2) + \frac{2k^2c_1^2d_2^2}{a^2b^2c^2}tr(C^2)\right]\sigma_e^4\\ &+ \left[\left(\frac{c_1}{a}tr(AV_1) - 1\right)\sigma_{u1}^2 + \left(\frac{c_1}{a}tr(AV_2) - \frac{dc_1d_1}{ab}tr(BV_2)\right)\right]\sigma_2^2\\ &+ \left[\frac{c_1}{a}tr(A) - \frac{dc_1d_1}{ab}tr(B) + \frac{c_1kd_2}{abc}tr(C)\sigma_e^2\right]^2. \qquad (32)\end{aligned}$$

We write the latter expression as below. First let

$$r = \frac{c_1}{a} tr(AV_2) - \frac{dc_1d_1}{ab} tr(BV_2).$$

Rewriting it gives the following:

$$r = \frac{c_1 d}{a} - \frac{dc_1d_1}{a},$$

where from (18) we have $tr(AV_2) = d$ and $tr(BV_2) = b$. Moreover, let

$$t = \frac{c_1}{a} tr(A) - \frac{dc_1d_1}{ab} tr(B) + \frac{c_1kd_2}{ab}. \tag{33}$$

Hence, the following mean square error is obtained for the modified estimator $\widehat{\sigma}^2_{u11}$:

$$\begin{aligned}
\mathrm{MSE}(\widehat{\sigma}^2_{11}) = & \left[\frac{2c_1^2}{a^2} tr(AV_1AV_1) + (c_1-1)^2\right] \sigma_1^4 \\
& + \left[\frac{4c_1^2}{a^2} tr(AV_1AV_2) + 2(c_1-1)r\right] \sigma_1^2\sigma_2^2 \\
& + \left[\frac{2c_1^2}{a^2} tr(AV_2AV_2) + \frac{2d^2c_1^2d_1^2}{a^2b^2} tr(BV_2BV_2) + r^2\right] \sigma_2^4 \\
& + \left[\frac{4c_1^2}{a^2} tr(A^2V_1) + 2(c_1-1)t\right] \sigma_1^2\sigma_e^2 \\
& + \left[\frac{4c_1^2}{a^2} tr(A^2V_2) + \frac{4d^2c_1^2d_1^2}{a^2b^2} tr(B^2V_2) + 2rt\right] \sigma_2^2\sigma_e^2 \\
& + \left[\frac{2c_1^2}{a^2} tr(A^2) + \frac{2d^2c_1^2d_1^2}{a^2b^2} tr(B^2) + \frac{2k^2c_1^2d_2^2}{a^2b^2c^2} tr(C^2) + t^2\right] \sigma_e^4. \quad (34)
\end{aligned}$$

3.1 Mean square error comparison

In this section we compare the mean square errors of the modified $\widehat{\sigma}^2_{11}$ and the unmodified estimator $\widehat{\sigma}^2_{u1}$, given by (34) and (25), respectively. We will investigate if $\mathrm{MSE}(\widehat{\sigma}^2_{11}) \leq \mathrm{MSE}(\widehat{\sigma}^2_{u1})$. To do so we compare all coefficients of σ_1^4, σ_2^4 and σ_e^4 and all their cross combinations which appeared in (34) and (25). We will investigate a number of inequalities. If they hold, then the coefficients of the modified estimator $\widehat{\sigma}^2_{11}$ are less than the coefficients of the unmodified one $\widehat{\sigma}^2_{u1}$.
From the terms corresponding to σ_1^4 in (34) and (25) it follows that we have to investigate if

$$\frac{2c_1^2}{a^2} \mathrm{tr}(AV_1AV_1) + (c_1-1)^2 \leq \frac{2}{a^2} \mathrm{tr}(AV_1AV_1). \tag{35}$$

From the terms corresponding to σ_2^4 we obtain that

$$\begin{aligned}&\frac{2c_1^2}{a^2}\mathrm{tr}(AV_2AV_2)+\frac{2d^2c_1^2d_1^2}{a^2b^2}\mathrm{tr}(BV_2BV_2)+r^2\\&\leq\frac{2}{a^2}\mathrm{tr}(AV_2AV_2)+\frac{2d^2}{a^2b^2}\mathrm{tr}(BV_2BV_2),\end{aligned}\tag{36}$$

should be studied, where $r=(\frac{c_1d}{a}-\frac{dc_1d_1}{a})$ and by assumption $c_1>0$. Corresponding to σ_e^4 we will study the inequality

$$\begin{aligned}&\frac{2c_1^2}{a^2}\mathrm{tr}(A^2)+\frac{2d^2c_1^2d_1^2}{a^2b^2}\mathrm{tr}(B^2)+\frac{2k^2c_1^2d_2^2}{a^2b^2c}+t^2\\&\leq\frac{2}{a^2}\mathrm{tr}(A^2)+\frac{2d^2}{a^2b^2}\mathrm{tr}(B^2)+\frac{2k^2}{a^2b^2c}\end{aligned}\tag{37}$$

where $k=d\mathrm{tr}(B)-b\mathrm{tr}(A)$ and t is defined in (33).
Now the cross combination coefficients of (25) and (34) will be compared. We have first the coefficients of $\sigma_1^2\sigma_2^2$.

$$\frac{4c_1^2}{a^2}\mathrm{tr}(AV_1AV_2)+2(c_1-1)r\leq\frac{4}{a^2}\mathrm{tr}(AV_1AV_2),\tag{38}$$

where

$$(c_1-1)r=(c_1-1)(\frac{c_1d}{a}-\frac{dc_1d_1}{a})=\frac{d}{a}(1-d_1)(c_1^2-c_1).$$

Corresponding to $\sigma_1^2\sigma_e^2$ we investigate

$$\frac{4c_1^2}{a^2}\mathrm{tr}(A^2V_1)+2(c_1-1)t\leq\frac{4}{a^2}tr(A^2V_1),\tag{39}$$

where

$$(c_1-1)t=(c_1-1)\left(\frac{c_1}{a}\mathrm{tr}(A)-\frac{dc_1d_1}{ab}tr(B)+\frac{c_1kd_2}{ab}\right),$$

and A, defined in (18), is an idempotent matrix. Finally we also study the coefficients corresponding to $\sigma_2^2\sigma_e^2$,

$$\frac{4c_1^2}{a^2}\mathrm{tr}(AV_2)+\frac{4d^2c_1^2d_1^2}{a^2b^2}tr(B^2V_2)+2rt\leq\frac{4}{a^2}\mathrm{tr}(A^2V_2)+\frac{4d^2}{a^2b^2}tr(B^2V_2),\tag{40}$$

where

$$2rt = 2\left(\frac{c_1 d}{a} - \frac{dc_1 d_1}{a}\right)\left(\frac{c_1}{a}\mathrm{tr}(A) - \frac{dc_1 d_1}{ab}\mathrm{tr}(B) + \frac{c_1 k d_2}{ab}\right)$$
$$= \frac{2c_1^2 d}{a^2}(1-d_1)\left(\mathrm{tr}(A) - \frac{dd_1}{b}\mathrm{tr}(B) + \frac{k}{b}d_2\right).$$

In order to find appropriate values of c_1, d_1 and d_2 we have chosen to minimize the leading terms in (34), i.e., the terms that involve the coefficients of σ_1^4, σ_2^4 and σ_e^4, respectively. When minimizing the coefficient of σ_1^4 in (34) the following equation is obtained,

$$\frac{\partial}{\partial c_1}\left[\frac{2c_1^2}{a^2}tr(AV_1AV_1) + (c_1-1)^2\right] = 0,$$

with a solution given by

$$c_1 = \frac{1}{\frac{2}{a^2}tr(AV_1AV_1)+1}. \tag{41}$$

Moreover, minimizing the coefficient of σ_2^4 gives

$$\frac{\partial}{\partial d_1}\left[\frac{2c_1^2}{a^2}tr(AV_2AV_2) + \frac{2d^2c_1^2d_1^2}{a^2b^2}tr(BV_2BV_2) + \left(\frac{c_1 d}{a} - \frac{dc_1 d_1}{a}\right)^2\right] = 0,$$

which implies

$$d_1 = \frac{1}{\frac{2}{b^2}tr(BV_2BV_2)+1}. \tag{42}$$

Finally, when minimizing the coefficient of the error variance component σ_e^4 we have to solve

$$\frac{\partial}{\partial d_2}\left[\frac{c_1^2}{a^2}tr(A^2) + \frac{2d^2c_1^2d_1^2}{a^2b^2}tr(B^2) + \frac{2k^2c_1^2d_2^2}{a^2b^2c}\right.$$
$$+\frac{c_1^2}{a^2}(tr(A))^2 - \frac{2dc_1^2d_1}{a^2b}tr(A)tr(B) + \frac{2c_1^2kd_2}{a^2b}tr(A)$$
$$\left.-\frac{2dc_1^2kd_1d_2}{a^2b^2}tr(B) + \frac{c_1^2k^2d_2^2}{a^2b^2} + \frac{d^2c_1^2d_1^2}{a^2b^2}(tr(B))^2\right] = 0.$$

The minimum is obtained when

$$d_2 = \frac{\frac{d}{b}d_1tr(B) - tr(A)}{(\frac{k}{b})(\frac{2}{c}+1)}. \tag{43}$$

It has been verified that if c_1, d_1 and d_2 satisfy the minimum of the coefficients σ_1^4, σ_2^4 and σ_e^4, respectively, in equation (34). It follows that (35) and (36) hold for the given values in (41) and (42), respectively. Concerning (37), omitting a^2 and simplifying, the left hand side can be written as

$$c_1^2 tr(A) + \frac{d^2 c_1^2 d_1^2}{b^2}\mathrm{tr}(B) + \frac{k^2 c_1^2 d_2^2}{b^2 c} + \frac{1}{2}c_1^2\left(\mathrm{tr}(A) - \frac{d}{b}d_1\mathrm{tr}(B) + \frac{kd_2}{b}\right)^2. \quad (44)$$

However, since c_1 and d_1 given by (41) and (42) respectively, are less than 1 it is enough to study when

$$\frac{k^2 c_1^2 d_2^2}{b^2 c} + \frac{1}{2}c_1^2\left(\mathrm{tr}(A) - \frac{d}{b}d_1\mathrm{tr}(B) + \frac{kd_2}{b}\right)^2 \leq \frac{k^2}{b^2 c} \quad (45)$$

The following is obtained after substituting d_2 defined in (43) into the left hand side of (45)

$$\frac{k^2 c_1^2}{b^2 c}\frac{(\frac{d}{b}d_1\mathrm{tr}(B) - \mathrm{tr}(A))^2}{(\frac{k}{b})^2(\frac{2}{c}+1)^2} + \frac{c_1^2}{2}\left(\mathrm{tr}(A) - \frac{d}{b}d_1 tr(B) + \frac{k}{b}\frac{\frac{d}{b}d_1 tr(B) - \mathrm{tr}(A)}{(\frac{k}{b})(\frac{2}{c}+1)}\right)^2 \quad (46)$$

which can be simplified to,

$$c_1^2\left(\frac{d}{b}d_1\mathrm{tr}(B) - \mathrm{tr}(A)\right)^2\left[\frac{c}{(2+c)^2} - \frac{2}{(2+c)^2}\right]. \quad (47)$$

Hence, for (37) to hold the following must be satisfied

$$\left(\frac{d}{b}d_1\mathrm{tr}(B) - \mathrm{tr}(A)\right)^2 \leq \left(\frac{d}{b}\mathrm{tr}(B) - \mathrm{tr}(A)\right)^2. \quad (48)$$

Therefore we have two cases to consider, either

$$\mathrm{tr}(A) \leq \frac{d}{b}d_1\mathrm{tr}(B), \quad (49)$$

or

$$\mathrm{tr}(A) > \frac{d}{b}d_1\mathrm{tr}(B), \quad (50)$$

which have to be treated separately. If (49) holds, then (48) is always satisfied. If instead (50) is true we will return one step and suppose $d_1 = 1$. Then, obviously (36) and (48) will hold. Observe that $d_1 = 1$ means that we should not perturb (26) with respect to d_1.
Moreover, (38) is always satisfied since,

$$(c_1 - 1)r = \frac{d}{a}(1 - d_1)(c_1^2 - c_1) \leq 0. \quad (51)$$

Concerning (39), we study the second term in the left hand side,

$$(c_1 - 1)t = (c_1 - 1)\left(\frac{c_1}{a}tr(A) - \frac{dc_1 d_1}{ab}tr(B) + \frac{c_1 kd_2}{ab}\right).$$

Substituting d_2, defined in (43), yields

$$(c_1-1)\left(\frac{c_1}{a}tr(A)-\frac{dc_1d_1}{ab}tr(B)+\frac{c_1k}{ab}\frac{\frac{d}{b}d_1tr(B)-tr(A)}{(\frac{k}{b})(\frac{2}{c}+1)}\right),$$

giving

$$\frac{1}{a}(c_1^2-c_1)\left(tr(A)-\frac{dc_1}{b}d_1tr(B)+\frac{\frac{d}{b}d_1tr(B)-tr(A)}{\frac{2}{c}+1}\right).$$

Thus, for (39), we have from (18) that $\mathrm{tr}(AV_1)=a$ which implies that (39) can be written as

$$\frac{2c_1^2}{a}+\frac{1}{a}(c_1^2-c_1)\left(tr(A)-\frac{dc_1}{b}d_1tr(B)+\frac{\frac{d}{b}d_1tr(B)-tr(A)}{\frac{2}{c}+1}\right)\leq\frac{2}{a}.$$

Hence, if (49) is true (39) will hold if

$$2c_1^2+(c_1^2-c_1)\left(\mathrm{tr}(A)-\frac{d}{b}d_1\mathrm{tr}(B)\right)\left(\frac{2}{2+c}\right)\leq 2, \tag{52}$$

and we obtain the additional condition

$$\mathrm{tr}(A)\geq\frac{d}{b}d_1\mathrm{tr}(B)-\frac{(2+c)(1+c_1)}{c_1}. \tag{53}$$

If (50) holds, then it's obvious that (53) will be true. Finally, we check the inequality (40). Since from (18) we have $\mathrm{tr}(AV_2)=d$ and $\mathrm{tr}(BV_2)=b$ we rewrite (40) as

$$\frac{4c_1^2d}{a^2}+\frac{4d^2c_1^2d_1^2}{a^2b^2}+\frac{2c_1^2d}{a^2}(1-d_1)\left(\mathrm{tr}(A)-\frac{dd_1}{b}\mathrm{tr}(B)+\frac{k}{b}d_2\right)\leq\frac{4d}{a^2}+\frac{4d^2}{a^2b}.$$

It is enough to investigate the third term in the left hand side:

$$\frac{c_1^2d}{a^2}(1-d_1)\left(\mathrm{tr}(A)-\frac{dd_1}{b}\mathrm{tr}(B)+\frac{k}{b}d_2\right).$$

As previously, after substituting d_2 and omitting identical terms from both sides, (40) can be written as,

$$2c_1^2+\frac{2dc_1^2d_1^2}{b}+c_1^2(1-d_1)\left(\mathrm{tr}(A)-\frac{d}{b}d_1\mathrm{tr}(B)\right)\left(\frac{2}{2+c}\right)\leq 2+\frac{2d}{b}. \tag{54}$$

Thus, (40) is satisfied under (49). Moreover, if $d_1=1$ as assumed if (50) holds, then (40) is also valid. The above results can be summarized in the following proposition

Proposition 1 Let the variance component estimator corresponding to the first random effect $\widehat{\sigma}^2_{u1}$ in the model defined in (11) be modified as in (27), where c_1, d_1 and d_2 are chosen as in (41), (42) and (43), respectively. Then (35)– (40) are sufficient conditions for $\mathrm{MSE}(\widehat{\sigma}^2_{11}) \leq \mathrm{MSE}(\widehat{\sigma}^2_{u1})$.

Moreover, for the two cases that emerged from (48) we have the following theorem

Theorem 1 Given the model defined in (11), let $\mathrm{MSE}(\widehat{\sigma}^2_{u1})$ be the mean square error of the unmodified estimator given in (25) and let $\mathrm{MSE}(\widehat{\sigma}^2_{11})$ be the mean square error of the modified estimator given in (34).

(i) If (49) and (53) hold, $\mathrm{MSE}(\widehat{\sigma}^2_{11}) \leq \mathrm{MSE}(\widehat{\sigma}^2_{u1})$.
(ii)If (50) and $d_1 = 1$, $\mathrm{MSE}(\widehat{\sigma}^2_{11}) \leq \mathrm{MSE}(\widehat{\sigma}^2_{u1})$.

4 Conclusion

The problem of modifying the variance component estimator obtained by using Henderson's method 3, has been the focus of our work.

För a two-way linear mixed model, consisting of three variance components, σ^2_1, σ^2_2, and σ^2_e, we have perturbed the Henderson's estimation equations. The main aim, was to modify the standard unbiased estimator, corresponding to one of the random effects, by multiplying the estimator with some coefficients that are chosen to minimize the leading terms σ^2_1, σ^2_2, and σ^2_e in the mean square error equation. Two modified variance component estimators are proposed; each appropriate under certain given conditions. Our proposed estimators are easy to compute and have smaller MSE than the unmodified one. Moreover, the conditions under which each of the proposed estimators are valid, are easy to investigate. For instance, in practical application if the unbiasedness condition is not of major concern, our proposed estimators should be considered.

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ORIGINAL ARTICLE

Non-iterative variance component estimation in QTL analysis

L. Rönnegård[1,*] R. Al-Sarraj[2] & D. von Rosen[2]

1 Linnaeus Centre for Bioinformatics, Uppsala University, Uppsala, Sweden
2 Centre of Biostochastics, Swedish University of Agricultural Sciences, Uppsala, Sweden

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Correspondence
L. Rönnegård, Linnaeus Centre for Bioinformatics, Uppsala University, SE-75124 Uppsala, Sweden.
Tel: +46 2377 8757; Fax: +46 2377 8054;
E-mail: lrn@du.se.

*Present address: Dalarna University, Rödavägen 3, SE-78170 Borlänge, Sweden.

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Summary

In variance component quantitative trait loci (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 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 with REML on data from a European wild boar × 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.

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 analysed using a simple regression model that assumes no genetic variation in QTL 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 modelled as a random effect (Perez-Enciso and Varona 2000; Rönnegård et al. 2008). 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 pedigree founders are assumed unrelated to genes randomly sampled from an outbred population (see Rönnegård et al. 2008 for models with related founders). Here, the QTL effects are modelled 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 (REML) 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 analysed, 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 non-negative 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 was 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 includes only 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.

Materials and methods

QTL variance component model

The aim of a QTL analysis is to detect regions most likely to harbour genes affecting the trait studied. In the data set that we analyse, 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

$$Y = X\beta + Z_1 u_1 + Z_2 u_2 + e, \quad (1)$$

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 Eqn (1), u_1, is an $m \times 1$ vector of independently normally distributed base generation allele effects, i.e. $u_1 \sim \text{MVN}(0, \frac{1}{2}\sigma_1^2 I)$where I is the identity matrix and σ_1^2 is the QTL genotypic variance. The number of base generation alleles m equals twice the number of base generation individuals. The QTL 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. $\text{cov}(u_i, u_j) = 0$ where i and j are different indices for the m base alleles. 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 Eqn (1) by u_2 is the $q \times 1$ vector of family effects, $u_2 \sim \text{MVN}(0, I\sigma_2^2)$ and Z_2 is the corresponding $n \times q$ incidence matrix. e is the $n \times 1$ vector of random error with $e \sim \text{MVN}(0, I\sigma_e^2)$ where σ_e^2 is the error variance. The variance–covariance matrix of Y is therefore:

$$V = \tfrac{1}{2} Z_1 Z_1' \sigma_1^2 + Z_2 Z_2' \sigma_2^2 + I\sigma_e^2 \quad (2)$$

where $0.5 Z_1 Z_1'$ is the identity-by-descent (IBD) matrix $\prod$. 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 $\prod$ are found in Fernando and Grossman (1989), Almasy and Blangero (1998) and 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 $\prod$.

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 as 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 variance component estimators obtained 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 an 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:

$$\hat{\sigma}_1^2 = c_1\left(\frac{Y'(P_{x1}-P_x)Y}{\mathrm{tr}(P_{x1}-P_x)V_1} - \frac{\mathrm{tr}(P_{x1}-P_x)V_2 d_1 Y'(P_{x_{12}}-P_{x1})Y}{\mathrm{tr}(P_{x1}-P_x)V_1\ \mathrm{tr}(P_{x_{12}}-P_{x1})V_2} + \frac{kd_2 Y'(I-P_{x_{12}})Y}{\mathrm{tr}(P_{x_{12}-P_{x1}})V_2\ \mathrm{tr}(I-P_{x_{12}})}\right). \quad (3)$$

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

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

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

Data

A detailed description of the data is found in Knott et al. (1998) and Lundström et al. (1995). In the analysed 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 ($\mathrm{Hal}^N/\mathrm{Hal}^n$) for this gene, whereas all other founders were homozygotes ($\mathrm{Hal}^N/\mathrm{Hal}^N$) 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.

Analysis

The standard method to analyse 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 partitions I and II. These were compared with 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: $\mathrm{LR} = -2(l_0 - l_1)$ where l_1 is the log-likelihood for Eqn (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 $\sigma_1^2 = 0$). Approximations of the LR curve were calculated by calculating the log-likelihood for $\hat{\sigma}_2^2$ and $\hat{\sigma}_e^2$ estimated under the null hypothesis and $\hat{\sigma}_1^2$ estimated with one of the modified Henderson's estimators. The approximated LR values were put equal to 0 for negative values of $\hat{\sigma}_1^2$.

Results

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

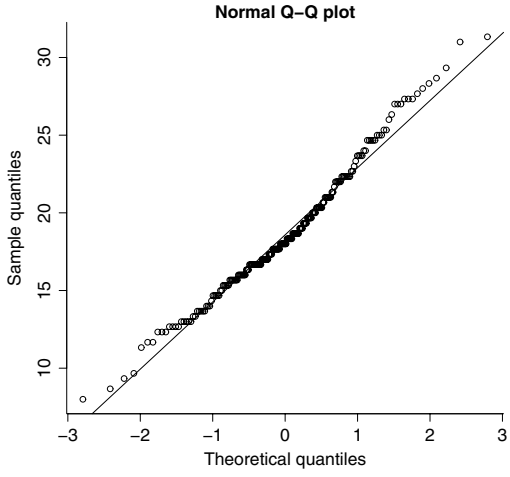

Figure 1 Q-Q-plot of the F2 individuals' meat quality (EEL) values from the studied Wild Boar x Domestic Pig intercross.

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 and 5.06. The modified partition I estimators resulted in slightly lower estimates and the difference was the 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, we could not conclude which of the two partitions that was the superior one.

Likelihood ratio curve

The LR curve, obtained from fitting the variance component QTL model in Eqn (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 $\hat{\sigma}_2^2 = 0.94$ and $\hat{\sigma}_e^2 = 17.6$.

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).

For positions with low LR values the uncertainty in the VC estimates are large. Consequently, the variation between the VC estimators in Figure 2 increases when we move away from the QTL position.

Discussion

We have tested a new non-iterative variance component estimation method on a QTL chromosome scan

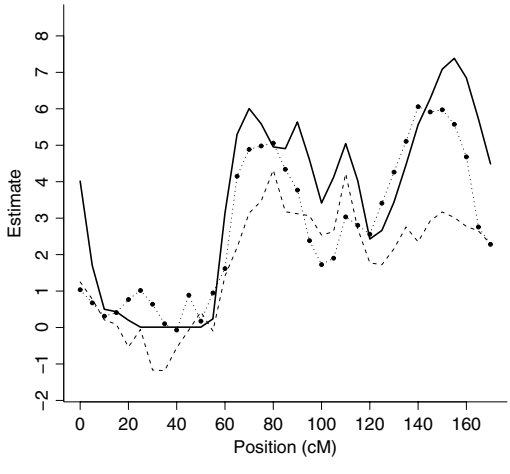

Figure 2 Estimates of the QTL variance σ_1^2 along pig chromosome 6. Solid line-REML estimates, dashed line-modified Partition I, dotted line with bullets-modified Partition II.

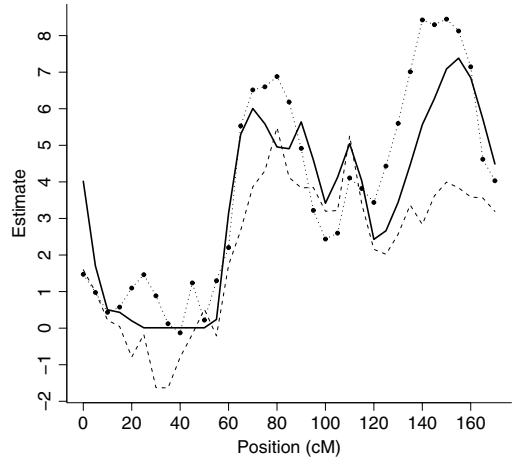

Figure 3 Non-modified estimators of the QTL variance σ_1^2 along pig chromosome 6. Solid line-REML estimators, dashed line-Partition I, dotted line with bullets-Partition II.

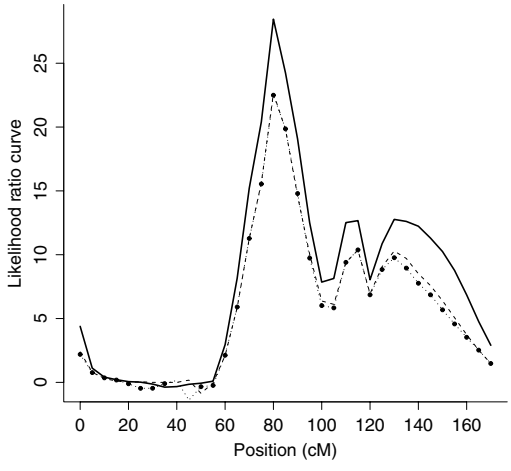

Figure 4 Likelihood ratio (LR) values along pig chromosome 6. The halothane gene affecting meat quality is located at 80 cM. LR values from REML given as solid line, approximated LR values from modified Partition I given as dashed line, and, approximated LR values from modified Partition II given as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

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 analysed pedigrees is likely to increase in future, making full genome scans computationally slow or even infeasible.

We present explicit solutions for QTL variance estimation and our main focus has been on comparing VC estimates. These explicit solutions 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, we define the following matrices $[X]$, $[X,Z_1]$ and $[X,Z_1,Z_2]$. The corresponding projection matrices are

$$P_x = X(X'X)^{-}X'$$
$$P_{x_1} = (X,Z_1)((X,Z_1)'(X,Z_1))^{-}(X,Z_1)'$$
$$P_{x_{12}} = (X,Z_1,Z_2)((X,Z_1,Z_2)'(X,Z_1,Z_2))^{-}(X,Z_1,Z_2)'$$

where $-$ represents the g-inverse $AA^{-}A = A$. The first set of estimation equation partition I are based on the following quadratic forms and their expectations:

$$\begin{pmatrix} Y'(P_{x_1}-P_x)Y \\ Y'(P_{x_{12}}-P_{x_1})Y \\ Y'(I-P_{x_{12}})Y \end{pmatrix} = E\begin{pmatrix} Y'(P_{x_1}-P_x)Y \\ Y'(P_{x_{12}}-P_{x_1})Y \\ Y'(I-P_{x_{12}})Y \end{pmatrix}$$

which gives a set of equations:

$$\begin{pmatrix} Y'(P_{x_1}-P_x)Y \\ Y'(P_{x_{12}}-P_{x_1})Y \\ Y'(I-P_{x_{12}})Y \end{pmatrix} = J\begin{pmatrix} \sigma^2_{u_1} \\ \sigma^2_{u_2} \\ \sigma^2_{u_e} \end{pmatrix}$$

where the elements in the matrix J are traces of matrix products.

The VC estimates for Henderson's method are then obtained as:

$$\begin{pmatrix} \hat{\sigma}^2_{u_1} \\ \hat{\sigma}^2_{u_2} \\ \hat{\sigma}^2_{u_e} \end{pmatrix} = J^{-1}\begin{pmatrix} Y'(P_{x_1}-P_x)Y \\ Y'(P_{x_{12}}-P_{x_1})Y \\ Y'(I-P_{x_{12}})Y \end{pmatrix}$$

The modified estimates are obtained from:

$$\begin{pmatrix} \hat{\sigma}^2_{u_1} \\ \hat{\sigma}^2_{u_2} \\ \hat{\sigma}^2_{u_e} \end{pmatrix} = J^{-1}\begin{pmatrix} c_1 Y'(P_{x_1}-P_x)Y \\ c_1 d_1 Y'(P_{x_{12}}-P_{x_1})Y \\ c_1 d_2 Y'(I-P_{x_{12}})Y \end{pmatrix}$$

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 the following quadratic forms and their expectations:

$$\begin{pmatrix} Y'(P_{x_{12}}-P_{x_2})Y \\ Y'(I-P_{x_{12}})Y \end{pmatrix} = E\begin{pmatrix} Y'(P_{x_{12}}-P_{x_2})Y \\ Y'(I-P_{x_{12}})Y \end{pmatrix}$$

which gives a set of equations:

$$\begin{pmatrix} Y'(P_{x_{12}}-P_{x_2})Y \\ Y'(I-P_{x_{12}})Y \end{pmatrix} = K\begin{pmatrix} \sigma^2_{u_1} \\ \sigma^2_{u_e} \end{pmatrix}$$

where the elements in the matrix K are traces of matrix products.

The modified estimates for partition II are obtained from:

$$\begin{pmatrix} \hat{\sigma}^2_{u_1} \\ \hat{\sigma}^2_{u_e} \end{pmatrix} = K^{-1}\begin{pmatrix} c_2 Y'(P_{x_{12}}-P_{x_2})Y \\ \varepsilon_1 Y'(I-P_{x_{12}})Y \end{pmatrix}$$

The coefficients c_1,d_1 and d_2 minimizing the mean square error of Eqn (3) are

$$c_1 = \frac{1}{\frac{2}{[\mathrm{tr}(P_{x1}-P_x)V_1]^2}[\mathrm{tr}(P_{x1}-P_x)V_1(P_{x1}-P_x)V_1]+1},$$

$$d_1 = \frac{1}{\frac{2}{[\mathrm{tr}(P_{x_{12}}-P_{x1})V_2]^2}[\mathrm{tr}(P_{x_{12}}-P_{x1})V_2(P_{x_{12}}-P_{x1})V_2]+1},$$

$$d_2 = \frac{\frac{(\mathrm{tr}(P_{x1}-P_x)V_2)}{\mathrm{tr}(P_{x_{12}}-P_{x1})V_2}d_1\mathrm{tr}(P_{x_{12}}-P_{x1})-\mathrm{tr}(P_{x1}-P_x)}{\left[\frac{k}{\mathrm{tr}(P_{x_{12}}-P_{x1})V_2}\right]\left[\frac{2}{\mathrm{tr}(I-P_{x_{12}})}+1\right]}.$$

where $V_1 = Z_1Z_1'$ and $V_2 = Z_2Z_2'$. 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 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,

$$c_2 = \frac{1}{\frac{2}{[\mathrm{tr}(P_{x_{12}}-P_{x2})V_1]^2}[\mathrm{tr}(P_{x_{12}}-P_{x2})V_1\ \mathrm{tr}(P_{x_{12}}-P_{x2})V_1]},$$

$$\varepsilon_1 = \frac{1}{\frac{2}{\mathrm{tr}(I-P_{x_{12}})}+1}.$$

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RESEARCH PAPER

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Generalized prediction intervals for treatment effects in random-effects models

Razaw Al-Sarraj[1] | Claudia von Brömssen[1] | Johannes Forkman[1,2]

[1]Department of Energy and Technology, Swedish University of Agricultural Sciences, Uppsala, Sweden

[2]Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Correspondence
Razaw Al-Sarraj, Department of Energy and Technology, Swedish University of Agricultural Sciences, Box 7032, 750 07 Uppsala, Sweden.
Email: razaw.al-sarraj@slu.se

Abstract

This article derives generalized prediction intervals for random effects in linear random-effects models. For balanced and unbalanced data in two-way layouts, models are considered with and without interaction. Coverage of the proposed generalized prediction intervals was estimated in a simulation study based on an agricultural field experiment. Generalized prediction intervals were compared with prediction intervals based on the restricted maximum likelihood (REML) procedure and the approximate methods of Satterthwaite and Kenward and Roger. The simulation study showed that coverage of generalized prediction intervals was closer to the nominal level 0.95 than coverage of prediction intervals based on the REML procedure.

KEYWORDS

generalized prediction intervals, random effects, random models, REML

1 | INTRODUCTION

Random-effects models are extensively used in many areas of research. The models consist of a single fixed intercept and independently normally distributed random effects. Analyzing these models requires estimating not only the variance component due to residual error, but also the variance components due to the random effects. For this problem, an array of ANOVA and likelihood-based variance component estimation methods are available (Searle, Casella, & McCulloch, 1992). The random effects are predicted by the best linear unbiased predictions (BLUPs), which can be obtained from the so called mixed model equations (Henderson, 1953). The BLUPs are characterized by the shrinkage property (James & Stein, 1961), which results in treatment means that are shrunk toward the overall mean. As a consequence, BLUP gives smaller mean squared error (MSE) than best linear unbiased estimation (BLUE), which is used when effect are assumed to be fixed. For prediction of a specific treatment mean, BLUP utilizes all available data, while BLUE uses only the observations of the specific treatment. For a thorough explanation of the advantages of BLUP, see Robinson (1991).

Forkman and Piepho (2013) showed that in analysis of randomized complete block experiments, it is preferable to model effects of treatments as random in order to minimize mean square error in prediction of differences between treatment means. This applies also when the number of blocks and experimental treatments are small and estimates of variances are uncertain. Their result suggests that random-effects models are useful for analysis of agricultural field experiments. However, they noted that when the between-treatments variance was estimated to be zero, prediction intervals for treatment effects degenerate at zero using maximum-likelihood methods. In small experiments or when there are many variance components, zero-estimates of variance components are common, so this is a major drawback from a practical point of view. When treatment effects are estimated to be zero, this indicates that the null hypothesis of no treatment effects is true. However, we may still want to compute prediction intervals for the treatment effects.

To account for drawbacks of standard inferential procedures, Tsui and Weerahandi (1989) and Weerahandi (1993) introduced generalized pivotal quantities (GPQs) and generalized confidence intervals (GCIs). Many authors have used GPQs for

hypotheses testing and interval estimation in analysis of experiments. For a one-way and two-way balanced layout, Weerahandi (1991) developed GPQ tests for variance components that are equivalent to usual F-tests. Zhou and Mathew (1994) proposed generalized P-values in testing hypotheses regarding variance components in balanced-mixed models when exact F tests do not exist. Ye and Wang (2009) computed and evaluated GCIs for intraclass correlation coefficients in a two-way random effects model with interaction.

For an unbalanced nested model, Li and Wang (2011) proposed a method for testing functions of variance components using generalized P-values. Burdick, Quirozb, and Iyer (2006) provided GCIs for parameters in the one-way random-effects model, for both balanced and unbalanced data. Iyer and Patterson (2002) and Hannig, Iyer, and Patterson (2006) provided general methods for constructing GPQs and studied asymptotic properties. Wang, Hannig, and Iyer (2012) proposed, for several distributions, a method to construct prediction intervals for future observations.

For the one-way random-effects model with balanced data: $y_{ij} = \mu + u_i + e_{ij}$, where $i = 1, ..., a$ and $j = 1, ..., n$, with $u_i \sim N(0, \sigma_A^2)$ and $e_{ij} \sim N(0, \sigma_e^2)$, Gamage, Mathew, and Weerahandi (2013) derived a generalized prediction interval (GPI) for the predictor $\mu + k(\bar{y}_{1.} - \mu)$, where μ is the overall mean, $\bar{y}_{1.}$ is the mean of the first treatment effect and k is the shrinkage factor: $k = n\sigma_A^2/(\sigma_e^2 + n\sigma_A^2)$. Yu, Zou, Carlsson, and Weerahandi (2015) considered the problem of nonnegative estimates of the variance components and proposed a generalized estimate of the BLUP.

The aim of this article is to derive and introduce GPIs for $\mu + u_1$, u_1, and $u_1 - u_2$, in random-effects models. We are particularly interested in these quantities, since in analysis of experiments they represent the mean of the first treatment, the effect of the first treatment, and the difference between the first and the second treatment, respectively. These factor levels are considered without loss of generality.

In Section 2, we describe the new method and provide equations for balanced and unbalanced one-way and two-way layouts. Section 3 reviews other methods for computing prediction intervals in random-effects models. In Section 4, through a simulation study, we investigate performance of the methods. In Section 5, an agricultural field experiment exemplifies the method, followed by a discussion in Section 6.

2 | THE GENERALIZED PREDICTION INTERVAL METHOD FOR RANDOM EFFECTS

The GPI method that is presented here can be used for any linear combination of the intercept and the random effects. Sections 2.1–2.6 consider six different one-way and two-way random-effects models. In these models, $\mu + u_1$, u_1, and $u_1 - u_2$ may, depending on the context, correspond to a treatment mean, a treatment effect and a difference between two treatment effects, respectively. These are the linear combinations that we will focus on, as they are important in analysis of experiments. For the computation of GPIs for $\mu + u_1$, u_1, and $u_1 - u_2$, our proposed method can be described as follows:

(i) Derive the joint bivariate normal distributions of $(\mu + u_1, \bar{Y}_{1.})$, $(u_1, \bar{Y}_{1.} - \bar{Y}_{..})$, and $(u_1 - u_2, \bar{Y}_{1.} - \bar{Y}_{2.})$, where $\bar{Y}_{1.}$, $\bar{Y}_{1.} - \bar{Y}_{..}$ and $\bar{Y}_{1.} - \bar{Y}_{2.}$ are averages specified in Sections 2.1–2.6. The joint distributions are provided in Appendix A.1.

(ii) Derive the conditional distributions of $(\mu + u_1|\bar{Y}_{1.})$, $(u_1|\bar{Y}_{1.} - \bar{Y}_{..})$, and $(u_1 - u_2|\bar{Y}_{1.} - \bar{Y}_{2.})$, following Appendix A.2. With regard to each of the models considered in Sections 2.1–2.6, the shrinkage factors needed for the computations are provided in Appendix A.3.

(iii) Construct GPQs for the expected value and the variance components (Tsui & Weerahandi, 1989; Burdick, Borror, & Montgomery, 2005).

(iv) Substitute the GPQs for the parameters in the conditional normal distributions obtained in (ii).

(v) Sample from the conditional distribution. For each sample, use new independent samples of the GPQs.

(vi) The limits of the $1 - \alpha$ GPIs are the $100(\alpha/2)$th and $100(1 - \alpha/2)$th percentiles of the empirical distributions obtained in (v).

2.1 | One-way random-effects model, balanced data

Consider the model

$$Y_{ij} = \mu + u_i + e_{ij}, \quad i = 1, \ldots, a; \quad j = 1, \ldots, n, \tag{1}$$

where $u_i \sim N(0, \sigma_A^2)$ and $e_{ij} \sim N(0, \sigma_e^2)$ independently distributed. Let $\bar{y}_{i.} = \sum_{j=1}^{n} y_{ij}/n$ and $\bar{y}_{..} = \sum_{i=1}^{a} \bar{y}_{i.}/a$, where y_{ij} is a realized value, that is, an observation of Y_{ij}. In the context of an experiment, a is the number of treatments, and n is the number of observations per treatment. The BLUP of u_1 is given by $k(\bar{y}_{1.} - \bar{y}_{..})$, where $k = n\sigma_A^2/(\sigma_e^2 + n\sigma_A^2)$ is the shrinkage factor. The variance components are usually estimated using the maximum likelihood method or the restricted maximum likelihood (REML) method (Patterson & Thompson, 1971). Denote the sum of squares by x_A and x_E, where $x_A = \Sigma_i \Sigma_j (\bar{y}_{i.} - \bar{y}_{..})^2$ and $x_E = \Sigma_i \Sigma_j (y_{ij} - \bar{y}_{i.})^2$. The statistics x_A and x_E are realized values of the random variables X_A, and X_E, respectively.

Let $U_A = X_A/(\sigma_e^2 + n\sigma_A^2)$, $U_E = X_E/\sigma_e^2$ and $Z = (\bar{Y}_{..} - \mu)/\sqrt{Var(\bar{Y}_{..})}$, where $\bar{Y}_{..} = \sum_{i=1}^{a} \bar{Y}_{i.}/a$, $\bar{Y}_{i.} = \sum_{j=1}^{n} Y_{ij}/n$ and $\text{Var}(\bar{Y}_{..}) = \sigma_A^2/a + \sigma_e^2/an$. Then $U_A \sim \chi^2_{a-1}$, $U_E \sim \chi^2_{a(n-1)}$ and $Z \sim N(0,1)$. When replacing the random variables X_A, X_E, and $\bar{Y}_{..}$ by their observed values x_A, x_E, and $\bar{y}_{..}$, respectively, the following GPQs are obtained:

$$G(\sigma_A^2) = \frac{x_A}{nU_A} - \frac{x_E}{nU_E}, \tag{2}$$

$$G(\sigma_e^2) = \frac{x_E}{U_E}, \tag{3}$$

$$G(\mu) = \bar{y}_{..} - Z\sqrt{\frac{G(\sigma_A^2)}{a} + \frac{G(\sigma_e^2)}{an}}. \tag{4}$$

We now show how GPIs can be computed for $\mu + u_1$, u_1, and $u_1 - u_2$ in model (1), using the proposed method. The joint distributions are provided in Appendix A.1. Substituting (2), (3), and (4) for σ_A^2, σ_e^2, and μ, respectively, and applying the equations of Appendix A.2 the following conditional distributions are obtained:

$$(\mu + u_1)|\bar{y}_{1.} \sim N(G(\mu) + \hat{k}_{11}(\bar{y}_{1.} - G(\mu)), \max(0, \hat{k}_{11} G(\sigma_e^2)/n)), \tag{5}$$

$$u_1|(\bar{y}_{1.} - \bar{y}_{..}) \sim N(\hat{k}_{11}(\bar{y}_{1.} - \bar{y}_{..}), \max(0, G(\sigma_A^2)(1 - \hat{k}_{11}(a-1)/a))), \tag{6}$$

$$(u_1 - u_2)|(\bar{y}_{1.} - \bar{y}_{2.}) \sim N(\hat{k}_{11}(\bar{y}_{1.} - \bar{y}_{2.}), \max(0, 2\hat{k}_{11} G(\sigma_e^2)/n)), \tag{7}$$

where the shrinkage factor $\hat{k}_{11}$ is specified in Appendix A.3. GPIs are easily obtained through simulation of these conditional distributions. For each sample of the conditional distributions, new independent samples of $G(\sigma_A^2)$, $G(\sigma_e^2)$, and $G(\mu)$ are used for the computation of the expected values and variances. The limits of the $1 - \alpha$ GPIs are the $100(\alpha/2)$-th and $100(1 - \alpha/2)$-th percentiles of the obtained empirical distributions.

2.2 | One-way random-effects model, unbalanced data

When the one-way layout is unbalanced, that is, when the data has unequal cell frequencies, $i = 1, \ldots, a; j = 1, \ldots, n_i$, let $\bar{y}_{i.} = \sum_{i=1}^{n_i} y_{ij}/n_i$ and $\bar{y}_{..} = \sum_{i=1}^{a} \bar{y}_{i.}/a$ be observations of $\bar{Y}_{i.} = \sum_{i=1}^{n_i} Y_{ij}/n_i$ and $\bar{Y}_{..} = \sum_{i=1}^{a} \bar{Y}_{i.}/a$, respectively. Further, $\bar{Y}_{i.} \sim N(\mu, \sigma_A^2 + \sigma_e^2/n_i)$ and $\bar{Y}_{..} \sim N(\mu, (\sigma_A^2/a + \sigma_e^2/a\tilde{n}_h))$, where $\tilde{n}_h = a/\sum_i (1/n_i)$ is the harmonic mean of the cell frequencies. The error and treatment sum of squares are $X_E = \sum_{i=1}^{a} \sum_{j=1}^{n_i} (Y_{ij} - \bar{Y}_{i.})^2$ and $X_A = \tilde{n}_h \sum_{i=1}^{a} (\bar{Y}_{i.} - \bar{Y}_{..})^2$, respectively.

The GPQs, $G(\sigma_A^2)$, $G(\sigma_e^2)$, and $G(\mu)$ are defined as in Section 2.1, but $\tilde{n}_h$ is used instead of n. In this case, $U_A \sim \chi^2_{a-1}$ approximately, however, U_A is not independent of σ_A^2 and σ_e^2, (Thomas & Hultquist, 1978). From the joint densities of Appendix A.1, the following conditional densities are derived:

$$(\mu + u_1)|\bar{y}_{1.} \sim N(G(\mu) + \hat{k}_{21}(\bar{y}_{1.} - G(\mu)), \max(0, \hat{k}_{21} G(\sigma_e^2)/n_1)), \tag{8}$$

$$u_1|(\bar{y}_{1.} - \bar{y}_{..}) \sim N(\hat{k}_{22}(\bar{y}_{1.} - \bar{y}_{..}), \max(0, G(\sigma_A^2)(1 - \hat{k}_{22}(a-1)/a))), \tag{9}$$

$$(u_1 - u_2)|(\bar{y}_{1.} - \bar{y}_{2.}) \sim N(\hat{k}_{23}(\bar{y}_{1.} - \bar{y}_{2.}), \max(0, 2G(\sigma_A^2)(1 - \hat{k}_{23}))), \tag{10}$$

where $G(\sigma_A^2)$, $G(\sigma_e^2)$, and $G(\mu)$ were substituted for σ_A^2, σ_e^2, and μ, respectively.

2.3 | Two-way random-effects model without interaction, balanced data

Consider the model

$$Y_{ij} = \mu + u_i + b_j + e_{ij}; \quad i = 1, \ldots, a; \quad j = 1, \ldots, b, \tag{11}$$

where $u_i \sim N(0, \sigma_A^2)$ are treatment effects, $b_j \sim N(0, \sigma_b^2)$ are block effects, and $e_{ij} \sim N(0, \sigma_e^2)$ are residual error effects. Let $\bar{Y}_{i.} = \sum_{j=1}^{b} Y_{ij}/b$ and $\bar{Y}_{..} = \sum_{i=1}^{a}\sum_{j=1}^{b} Y_{ij}/(ab)$. Then $\bar{Y}_{..} \sim N(\mu, \sigma_A^2/a + \sigma_b^2/b + \sigma_e^2/(ab))$. The sums of squares, denoted by x_A, x_B, and x_E are realized values of X_A, X_B, and X_E, respectively. Let $U_A = X_A/(\sigma_e^2 + b\sigma_A^2)$, $U_B = X_B/(\sigma_e^2 + a\sigma_b^2)$, $U_E = X_E/\sigma_e^2$, and $Z = (\bar{Y}_{..} - \mu)/\sqrt{Var(\bar{Y}_{..})}$, such that $U_A \sim \chi^2_{a-1}$, $U_B \sim \chi^2_{b-1}$, $U_E \sim \chi^2_{(a-1)(b-1)}$ and $Z \sim N(0,1)$. Replacing X_A, X_B, X_E, and $\bar{Y}_{..}$ by their realized values, the following GPQs are obtained:

$$G(\sigma_A^2) = \frac{x_A}{bU_A} - \frac{G(\sigma_e^2)}{b}, \tag{12}$$

$$G(\sigma_b^2) = \frac{x_B}{aU_B} - \frac{G(\sigma_e^2)}{a}, \tag{13}$$

$$G(\sigma_e^2) = \frac{x_E}{U_E}, \tag{14}$$

$$G(\mu) = \bar{y}_{..} - Z\sqrt{Var(\bar{y}_{..})}. \tag{15}$$

Let $\bar{y}_{i.}$ be the mean of the i-th treatment, and $\bar{y}_{..}$ the overall mean. The joint densities in Appendix A.1 are used to obtain the conditional distributions of $\mu + u_1$, u_1, and $u_1 - u_2$. When the parameters σ_A^2, σ_b^2, σ_e^2, and μ are replaced by their corresponding GPQs (12)–(15), these distributions can be written:

$$(\mu + u_1)|\bar{y}_{1.} \sim N(G(\mu) + \hat{k}_{31}(\bar{y}_{1.} - G(\mu)), \max(0, G(\sigma_A^2)(1 - \hat{k}_{31}))), \tag{16}$$

$$u_1|(\bar{y}_{1.} - \bar{y}_{..}) \sim N(\hat{k}_{32}(\bar{y}_{1.} - \bar{y}_{..}), \max(0, G(\sigma_A^2)(1 - \hat{k}_{32}(a-1)/a))), \tag{17}$$

$$(u_1 - u_2)|(\bar{y}_{1.} - \bar{y}_{2.}) \sim N(\hat{k}_{32}(\bar{y}_{1.} - \bar{y}_{2.}), \max(0, 2G(\sigma_A^2)(1 - \hat{k}_{32}))). \tag{18}$$

2.4 | Two-way random-effects model without interaction, unbalanced data

For the unbalanced case of model (11), that is, when $i = 1, \ldots a$, $j = 1 \ldots n_i$, and $N = \sum_{i=1}^{a} n_i$, let $\bar{y}_{i.} = \sum_{j=1}^{n_i} y_{ij}/n_i$ and $\bar{y}_{..} = \sum_{i=1}^{a} \bar{y}_{i.}/a$. Then $Var(\bar{y}_{i.}) = \sigma_A^2 + \sigma_b^2/n_i + \sigma_e^2/n_i$. Let c_{pq} be the number of blocks that include both treatments p and q. Thus $c_{pq} = n_p$ when $p = q$, and the matrix c_{pq} is the concurrence matrix (John & Williams, 1995, p.21). If $c_{pq} > 0$, let $d_{pq} = 1/c_{pq}$, otherwise let $d_{pq} = 0$. Then $Var(\bar{y}_{..}) = \sigma_A^2/a + \sigma_b^2 \sum_{p=1}^{a}\sum_{q=1}^{a} d_{pq}/a^2 + \sigma_e^2/(a\tilde{n}_h)$, where $\tilde{n}_h$ is the harmonic mean of $n_1, \ldots, n_a$. For the GPIs, the following random variables are defined: $U_A = X_A/(\sigma_e^2 + m_a\sigma_A^2)$, $U_B = X_B/(\sigma_e^2 + m_b\sigma_b^2)$, $U_E = X_E/\sigma_e^2$, and $Z = (\bar{Y}_{..} - \mu)/\sqrt{Var(\bar{Y}_{..})}$, where X_A, X_B and X_E are the Type III sums of squares (Henderson, 1953). The values of m_a and m_b are calculated as in Appendix A.4. Then $U_E \sim \chi^2_{(N-a-b+1)}$ and $Z \sim N(0,1)$. Moreover, $U_A \sim \chi^2_{a-1}$ and $U_B \sim \chi^2_{b-1}$, approximately. Replacing X_A, X_B, X_E, and $\bar{Y}_{..}$ by their observed values, the GPQs can be written as:

$$G(\sigma_A^2) = \frac{x_A}{m_a U_A} - \frac{G(\sigma_e^2)}{m_a}, \tag{19}$$

$$G(\sigma_b^2) = \frac{x_B}{m_b U_B} - \frac{G(\sigma_e^2)}{m_b}, \tag{20}$$

$$G(\sigma_e^2) = \frac{x_E}{U_E}, \tag{21}$$

$$G(\mu) = \bar{y}_{..} - Z\sqrt{Var(\bar{y}_{..})}. \tag{22}$$

For the GPIs, the joint densities, specified in Appendix A.1, are used to derive the conditional distributions:

$$(\mu + u_1)|\bar{y}_{1.} \sim N(G(\mu) + \hat{k}_{41}(\bar{y}_{1.} - G(\mu)), \max(0, G(\sigma_A^2)(1 - \hat{k}_{41}))), \tag{23}$$

$$u_1|(\bar{y}_{1.} - \bar{y}_{..}) \sim N(\hat{k}_{42}(\bar{y}_{1.} - \bar{y}_{..}), \max(0, G(\sigma_A^2)(1 - \hat{k}_{42}(a-1)/a))), \tag{24}$$

$$(u_1 - u_2)|(\bar{y}_{1\cdot} - \bar{y}_{2\cdot}) \sim N(\hat{k}_{43}(\bar{y}_{1\cdot} - \bar{y}_{2\cdot}), \max(0, 2G(\sigma_A^2)(1 - \hat{k}_{43}))). \tag{25}$$

2.5 | Two-way random-effects model with interaction, balanced data

Consider the model

$$Y_{ijr} = \mu + u_i + b_j + \gamma_{ij} + e_{ijr} \quad i = 1, \ldots, a; \quad j = 1, \ldots, b; \quad r = 1, \ldots, n. \tag{26}$$

It is assumed that $u_i \sim N(0, \sigma_A^2)$, $b_j \sim N(0, \sigma_b^2)$, $\gamma_{ij} \sim N(0, \sigma_{ab}^2)$ and $e_{ijr} \sim N(0, \sigma_e^2)$. The overall mean is $\bar{Y}_{\cdots} = \sum_{ijr} Y_{ijr}/(abn)$ with variance $\sigma_A^2/a + \sigma_b^2/b + \sigma_{ab}^2/(ab) + \sigma_e^2/(abn)$), and the i-th treatment mean is $\bar{Y}_{i\cdot\cdot} = \sum_{j=1}^{b} \sum_{r=1}^{n} Y_{ijr}/(bn)$ with variance $\sigma_a^2 + (\sigma_b^2 + \sigma_{ab}^2 + \sigma_e^2/n)/b$. The sums of squares for this model are denoted by x_A, x_B, $x_{AB,}$ and x_E. These are the realized values of the random variables X_A, X_B, $X_{AB,}$ and X_E, respectively. Let $U_A = X_A/(\sigma_e^2 + bn\sigma_A^2 + n\sigma_{ab}^2)$, $U_B = X_B/(\sigma_e^2 + an\sigma_b^2 + n\sigma_{ab}^2)$, $U_{AB} = X_{AB}/(\sigma_e^2 + n\sigma_{ab}^2)$, $U_E = X_E/\sigma_e^2$, and $Z = (\bar{Y}_{\cdots} - \mu)/\sqrt{Var(\bar{Y}_{\cdots})}$, such that $U_A \sim \chi_{a-1}^2$, $U_B \sim \chi_{b-1}^2$, $U_{AB} \sim \chi_{(a-1)(b-1)}^2$, $U_E \sim \chi_{(N-ab),}^2$ and $Z \sim N(0, 1)$. Replacing X_A, X_B, X_{AB}, X_E, and $\bar{Y}_{\cdot\cdot}$ by their realized values, the GPQs are:

$$G(\sigma_A^2) = \frac{x_A}{bnU_A} - \frac{G(\sigma_e^2)}{bn} - \frac{G(\sigma_{ab}^2)}{b} = \frac{1}{bn}\left(\frac{x_A}{U_A} - \frac{x_{AB}}{U_{AB}}\right). \tag{27}$$

$$G(\sigma_b^2) = \frac{x_B}{anU_B} - \frac{G(\sigma_e^2)}{an} - \frac{G(\sigma_{ab}^2)}{a} = \frac{1}{an}\left(\frac{x_B}{U_B} - \frac{x_{AB}}{U_{AB}}\right). \tag{28}$$

$$G(\sigma_{ab}^2) = \frac{x_{AB}}{nU_{AB}} - \frac{x_E}{nU_E}. \tag{29}$$

$$G(\sigma_e^2) = \frac{x_E}{U_E}. \tag{30}$$

$$G(\mu) = \bar{y}_{\cdots} - Z\sqrt{Var(\bar{y}_{\cdots})}. \tag{31}$$

The conditional distributions of the random effects are obtained using the joint densities given in Appendix A.1. Substituting the GPQs (27)–(31) for their corresponding parameters in the conditional distributions, the following conditional distributions are obtained:

$$(\mu + u_1)|\bar{y}_{1\cdot\cdot} \sim N(G(\mu) + \hat{k}_{51}(\bar{y}_{1\cdot\cdot} - G(\mu)), \max(0, G(\sigma_A^2)(1 - \hat{k}_{51}))), \tag{32}$$

$$u_1|(\bar{y}_{1\cdot\cdot} - \bar{y}_{\cdots}) \sim N(\hat{k}_{52}(\bar{y}_{1\cdot\cdot} - \bar{y}_{\cdots}), \max(0, G(\sigma_A^2)(1 - \hat{k}_{52}(a-1)/a))), \tag{33}$$

$$(u_1 - u_2)|(\bar{y}_{1\cdot\cdot} - \bar{y}_{2\cdot\cdot}) \sim N(\hat{k}_{52}(\bar{y}_{1\cdot\cdot} - \bar{y}_{2\cdot\cdot}), \max(0, 2G(\sigma_A^2)(1 - \hat{k}_{52}))). \tag{34}$$

2.6 | Two-way random-effects model with interaction, unbalanced data

For the model

$$Y_{ijr} = \mu + u_i + b_j + \gamma_{ij} + e_{ijr}; \quad i = 1, \ldots, a; \quad j = 1, \ldots, b, \quad r = 1, \ldots, n_{ij}, \tag{35}$$

where the parameters and random effects are defined as in (26), let $\bar{Y}_{ij\cdot} = \sum_{r=1}^{n_{ij}} Y_{ijr}/n_{ij}$, $\bar{Y}_{i\cdot\cdot} = \sum_{j=1}^{b} \bar{Y}_{ij\cdot}/b$ and $\bar{Y}_{\cdots} = \sum_{i=1}^{a} \sum_{j=1}^{b} \bar{Y}_{ij\cdot}/(ab)$. Further, let $\tilde{n}_h = ab/\sum_i \sum_j (1/n_{ij})$ denote the harmonic mean of all cell frequencies, and $\tilde{n}_{ih} = b/\sum_{j=1}^{b}(1/n_{ij})$ the harmonic means of the cell frequencies of the i-th treatment. Let X_A, X_B, and X_{AB} represent the unweighted sum of squares (Khuri, 1998) corresponding to u_i, b_j, and γ_{ij}, respectively. The random variables U_A, U_B, $U_{AB,}$ and U_E are defined as in Section 2.5, but n is replaced with the harmonic mean $\tilde{n}_h$. The random variables U_A, U_B, and U_{AB} are approximately chi-squared distributed with $a - 1$, $b - 1$, and $(a - 1)(b - 1)$ degrees of freedom, respectively (Khuri, 1998). Further, $U_E \sim \chi_{N-ab}^2$ and $Z \sim N(0, 1)$. The GPQs are obtained when replacing $\bar{Y}_{\cdots}$, X_A, X_B, $X_{AB,}$ and X_E by their observed values $\bar{y}_{\cdots}$,

x_A, x_B, x_{AB}, and x_E, respectively. The obtained GPQs are the same as the ones obtained in Section 2.5, but $\tilde{n}_h$ is used instead of n in (27)–(29). The conditional distributions are

$$(\mu + u_1)|\bar{y}_{1..} \sim N(G(\mu) + \hat{k}_{61}(\bar{y}_{1..} - G(\mu)), \max(0, G(\sigma_A^2)(1 - \hat{k}_{61}))), \tag{36}$$

$$u_1|(\bar{y}_{1..} - \bar{y}_{...}) \sim N(\hat{k}_{62}(\bar{y}_{1..} - \bar{y}_{...}), \max(0, G\left(\sigma_A^2\right)(1 - \hat{k}_{62}(a-1)/a))), \tag{37}$$

$$(u_1 - u_2)|(\bar{y}_{1..} - \bar{y}_{2..}) \sim N(\hat{k}_{63}(\bar{y}_{1..} - \bar{y}_{2..}), \max(0, 2G\left(\sigma_A^2\right)(1 - \hat{k}_{63}))). \tag{38}$$

3 | OTHER METHODS

3.1 | REML-based prediction intervals

In general, any random-effects model, including those discussed in Sections 2.1–2.6, can be written as:

$$\mathbf{Y} = \mathbf{X}\mu + \mathrm{Z}\mathbf{u} + \mathbf{e}, \tag{39}$$

where $\mathbf{Y}$ is an N-vector of observations, $\mathbf{X} = 1_N$ is an N-vector of ones, μ is a scalar, Z is an $N \times q$ incidence matrix, and $\mathbf{u}$ is a q-vector of unknown random effects that are distributed as $\mathbf{u} \sim N(\mathbf{0}, \mathrm{G})$. The N-vector of random errors $\mathbf{e}$ is distributed as $\mathbf{e} \sim N(\mathbf{0}, \mathrm{R})$. It follows that $\mathbf{Y} \sim N(\mathbf{X}\mu, \mathrm{ZGZ}^\top + \mathrm{R}$. The elements of the variance matrices G and R are unknown variance components that can be estimated using the REML method. Let $\mathbf{v} = (\mu, \mathbf{u}^\top)$. A REML-based prediction interval for $\hat{u} = \mathbf{vL}$, where $\mathbf{L}$ is a contrast vector, is computed as follows (Pawitan, 2001):

Step 1: The estimates μ and $\mathbf{u}$, denoted as $\hat{\mu}$ and $\hat{\mathbf{u}}$, respectively, are obtained by solving the mixed model equations (Henderson, 1975):

$$\begin{pmatrix} \mathbf{X}^\top \mathrm{R}^{-1}\mathbf{X} & \mathbf{X}^\top \mathrm{R}^{-1}\mathrm{Z} \\ \mathbf{Z}^\top \mathrm{R}^{-1}\mathbf{X} & \mathrm{Z}^\top \mathrm{R}^{-1}\mathrm{Z} + \mathrm{G}^{-1} \end{pmatrix} \begin{pmatrix} \hat{\mu} \\ \hat{\mathbf{u}} \end{pmatrix} = \mathrm{C} \begin{pmatrix} \hat{\mu} \\ \hat{\mathbf{u}} \end{pmatrix} = \begin{pmatrix} \mathbf{X}^\top \mathrm{R}^{-1}\mathbf{Y} \\ \mathrm{Z}^\top \mathrm{R}^{-1}\mathbf{Y} \end{pmatrix}.$$

Solving the equations above include calculating the generalized inverse of the covariance matrix denoted as C. This requires substituting in C the estimated values of G and R. The estimated value of C can be denoted as $\hat{\mathrm{C}}$.

Step 2: The standard error, denoted as $se(\hat{u})$, is calculated as $se(\hat{u}) = \sqrt{\mathbf{L}^\top \hat{\mathrm{C}} \mathrm{L}}$.

Step 3: The prediction interval for the different random effects are computed as:

$$\hat{u} \pm z_{\alpha/2}\, se(\hat{u}), \tag{40}$$

where $z_{\alpha/2}$ denotes the $100(1 - \alpha/2)$th percentile of the standard normal distribution.

3.2 | Approximate t-distributions

Instead of using the standard normal distribution in *Step* 3 of the REML-based method, approximate t-distributions can be used. The number of degrees of freedom, υ, can be computed using the so called containment method, the Satterthwaite (1946) method or the Kenward and Roger (1997) method. The containment method is the default using the mixed procedure of the SAS software package (Littell et al., 2006). The Kenward and Roger (1997) method applies the Satterthwaite approximation after an adjusment of the variance-covariance matrix. The prediction interval is calculated as $\hat{u} \pm t_\upsilon\, se(\hat{u})$, where $\hat{u}$ and $se(\hat{u})$ are defined as in Section 3.1, and t_υ denotes the $100(1 - \alpha/2)$-th percentile of the t-distribution with υ degrees of freedom.

3.3 | Gamage et al. (2013) generalized prediction intervals

For the one-way random-effects model (1), Gamage et al. (2013) considered the predictor $\mu_1^* = \mu + k(\bar{y}_{1.} - \mu)$, where k is the shrinkage factor $n\sigma_A^2/(\sigma_e^2 + n\sigma_A^2)$, and proposed using the percentiles of

$$\frac{G\left(\sigma_e^2\right)G(\mu) + nG(\sigma_A^2)\bar{y}_{1.}}{G\left(\sigma_e^2\right) + nG\left(\sigma_A^2\right)}, \tag{41}$$

TABLE 1 Estimated coverage of approximate 0.95 prediction intervals for $\mu + u_1$, u_1, and $u_1 - u_2$ in the one-way model, balanced data, using the REML-based method and the GPI method

			REML			GPI		
n	σ_e^2	σ_A^2	$\mu + u_1$	u_1	$u_1 - u_2$	$\mu + u_1$	u_1	$u_1 - u_2$
5	4	1	0.832	0.675	0.649	0.946	0.949	0.927
10	4	1	0.863	0.777	0.765	0.940	0.951	0.926
50	4	1	0.932	0.888	0.922	0.944	0.955	0.942
5	1	1	0.892	0.843	0.848	0.942	0.953	0.930
10	1	1	0.919	0.880	0.899	0.941	0.953	0.934
50	1	1	0.944	0.890	0.946	0.947	0.953	0.950
5	1	4	0.926	0.889	0.923	0.947	0.953	0.944
10	1	4	0.939	0.888	0.934	0.947	0.952	0.945
50	1	4	0.947	0.883	0.950	0.947	0.952	0.952

TABLE 2 Estimated coverage of Gamage et al. (2013) generalized 0.95 prediction intervals for μ_1^* and $\mu + u_1$ in the one-way model, balanced data

a	n	σ_e^2	σ_A^2	k	μ_1^*	$\mu + u_1$
5	5	4	1	0.556	0.949	0.717
5	10	4	1	0.714	0.954	0.602
5	50	4	1	0.926	0.951	0.341
5	5	1	1	0.833	0.949	0.513
5	10	1	1	0.909	0.951	0.389
5	50	1	1	0.980	0.950	0.192
5	5	1	4	0.950	0.951	0.320
5	10	1	4	0.975	0.951	0.220
5	50	1	4	0.995	0.951	0.090

where $G(\sigma_A^2)$, $G(\sigma_e^2)$, and $G(\mu)$ are given in (2), (3), and (4), respectively, for computation of GPIs. The 2.5th and 97.5th percentiles, obtained through sampling from (41), are the lower and upper limits of their 0.95 GPI for μ_1^*. Note that (41) is identically equal to the expected value of (5). Thus, GPIs for μ_1^* and $\mu + u_1$ are obtained through sampling from (41) and (5), respectively.

4 | SIMULATION STUDY

With regard to each of the six models considered, coverage of the generalized prediction intervals introduced in Section 2 was estimated using Monte Carlo sampling. The simulations in Tables 1–5 were carried out using the `lmer` function in `R` (`http://www.r-project.org`). For comparison, coverage was estimated for the REML-based prediction interval described in Section 3.1. Coverage was estimated as the frequency of 0.95 prediction intervals covering $\mu + u_1$, u_1, and $u_1 - u_2$.

The balanced one-way random-effects model was studied for the same cases, that is, the same combinations of the parameters a, n, σ_e^2, and σ_A^2, as studied by Gamage et al. (2013), using $\mu = 100$ (Table 1). In addition, for the same cases and using $\mu = 0$, coverage of the Gamage et al. (2013) prediction intervals (41) was studied, considering both μ_1^*, as defined in Section 3.3, and $\mu + u_1 = u_1$ (Table 2). In the unbalanced one-way random-effects model six different layouts were adopted; the first two of them are from Gamage et al. (2013) (Table 3).

The two-way random-effects models were studied for cases based on the oats variety trial that is presented in Section 5. In these cases, the model parameters were $a = 10$, $b = 4$, $\mu = 68$, $\sigma_b^2 = 15$, and $\sigma_e^2 = 24$. Several values of σ_A^2 were investigated, such that $k = n\sigma_A^2/(\sigma_e^2 + n\sigma_A^2)$ varied from 0 to 0.90. In model (26), the number of replicates per cell was $n = 3$ and $\sigma_{ab}^2 = 1$. In the unbalanced cases, 10% of the observations were randomly excluded, using the restrictions $n_i > 0$ for the model without interaction (Table 4) and $n_{ij} > 0$ for the model with interaction (Table 5).

TABLE 3 Estimated coverage of approximate 0.95 prediction intervals for $\mu + u_1$, u_1, and $u_1 - u_2$ in the one-way model, unbalanced data, using the REML-based method and the GPI method

			REML			GPI		
n_i	σ_e^2	σ_A^2	$\mu + u_1$	u_1	$u_1 - u_2$	$\mu + u_1$	u_1	$u_1 - u_2$
7,4,6,3	4	1	0.869	0.624	0.598	0.982	0.935	0.971
7,4,6,3	1	1	0.891	0.782	0.792	0.961	0.958	0.941
7,4,6,3	1	4	0.918	0.843	0.897	0.950	0.965	0.941
3,6,4,7	4	1	0.818	0.618	0.603	0.931	0.934	0.927
3,6,4,7	1	1	0.850	0.782	0.787	0.932	0.960	0.928
3,6,4,7	1	4	0.904	0.848	0.889	0.939	0.969	0.942
7,6,3,4	4	1	0.865	0.627	0.604	0.981	0.936	0.980
7,6,3,4	1	1	0.889	0.783	0.796	0.960	0.958	0.958
7,6,3,4	1	4	0.917	0.839	0.897	0.948	0.964	0.946
7,7,3,3	4	1	0.868	0.613	0.597	0.983	0.934	0.982
7,7,3,3	1	1	0.889	0.774	0.792	0.964	0.957	0.959
7,7,3,3	1	4	0.917	0.835	0.894	0.951	0.964	0.947
3,4,6,7	4	1	0.820	0.620	0.605	0.928	0.932	0.915
3,4,6,7	1	1	0.847	0.781	0.785	0.930	0.958	0.921
3,4,6,7	1	4	0.902	0.849	0.888	0.939	0.971	0.937
3,3,7,7	4	1	0.816	0.610	0.598	0.933	0.933	0.910
3,3,7,7	1	1	0.848	0.773	0.778	0.930	0.959	0.920
3,3,7,7	1	4	0.900	0.843	0.884	0.941	0.970	0.937

TABLE 4 Estimated coverage of approximate 0.95 prediction intervals for $\mu + u_1$, u_1, and $u_1 - u_2$ in the two-way model without interaction, using the REML-based method and the GPI method

		REML			GPI		
k	σ_A^2	$\mu + u_1$	u_1	$u_1 - u_2$	$\mu + u_1$	u_1	$u_1 - u_2$
Without interaction, balanced							
0	0	0.882	0.592	0.587	0.978	0.956	0.955
0.25	2	0.876	0.607	0.600	0.969	0.946	0.938
0.40	4	0.875	0.703	0.698	0.961	0.937	0.933
0.50	6	0.880	0.765	0.759	0.955	0.936	0.932
0.60	9	0.890	0.826	0.821	0.951	0.939	0.932
0.70	14	0.900	0.875	0.869	0.947	0.942	0.933
0.75	18	0.906	0.896	0.891	0.945	0.945	0.936
0.80	24	0.912	0.910	0.907	0.943	0.948	0.938
0.85	34	0.916	0.921	0.920	0.942	0.952	0.940
0.90	54	0.920	0.929	0.928	0.943	0.954	0.942
Without interaction, unbalanced							
0	0	0.882	0.585	0.580	0.973	0.964	0.982
0.25	2	0.874	0.573	0.570	0.967	0.947	0.964
0.40	4	0.871	0.663	0.660	0.964	0.944	0.947
0.50	6	0.876	0.730	0.724	0.963	0.944	0.939
0.60	9	0.884	0.792	0.789	0.958	0.947	0.937
0.70	14	0.893	0.847	0.849	0.958	0.954	0.934
0.75	18	0.901	0.872	0.871	0.958	0.958	0.935
0.80	24	0.907	0.891	0.892	0.955	0.962	0.934
0.85	34	0.913	0.906	0.908	0.955	0.968	0.936
0.90	54	0.918	0.917	0.921	0.956	0.974	0.935

TABLE 5 Estimated coverage of approximate 0.95 prediction intervals for $\mu + u_1$, u_1, and $u_1 - u_2$ in the two-way model with interaction, using the REML-based method and the GPI method

		REML			GPI		
k	σ_A^2	$\mu + u_1$	u_1	$u_1 - u_2$	$\mu + u_1$	u_1	$u_1 - u_2$
With interaction, balanced							
0	0	0.540	0.431	0.431	0.955	0.937	0.936
0.25	2	0.700	0.738	0.742	0.951	0.910	0.902
0.40	4	0.793	0.860	0.869	0.948	0.929	0.921
0.50	6	0.842	0.911	0.921	0.947	0.938	0.930
0.60	9	0.885	0.945	0.960	0.947	0.946	0.937
0.70	14	0.919	0.962	0.980	0.948	0.949	0.941
0.75	18	0.934	0.967	0.986	0.949	0.950	0.943
0.80	24	0.948	0.969	0.991	0.951	0.951	0.944
0.85	34	0.959	0.965	0.996	0.952	0.953	0.946
0.90	54	0.970	0.949	0.998	0.954	0.953	0.946
With interaction, unbalanced							
0	0	0.561	0.430	0.430	0.959	0.937	0.935
0.25	2	0.708	0.715	0.715	0.954	0.917	0.907
0.40	4	0.793	0.846	0.852	0.952	0.931	0.924
0.50	6	0.842	0.900	0.912	0.952	0.941	0.931
0.60	9	0.886	0.938	0.954	0.953	0.948	0.941
0.70	14	0.920	0.961	0.977	0.951	0.949	0.942
0.75	18	0.936	0.966	0.986	0.953	0.956	0.943
0.80	24	0.950	0.968	0.991	0.952	0.957	0.949
0.85	34	0.960	0.965	0.995	0.955	0.955	0.948
0.90	54	0.969	0.951	0.997	0.957	0.957	0.947

For each case, 10,000 datasets were randomly generated. For each generated dataset, GPIs were computed using 10,000 Monte-Carlo samples from the conditional distributions.

4.1 | Results

For the balanced one-way random-effects model (1), Table 1 shows estimated coverage for the REML-based and GPI methods, applying the percentiles of (40) for the REML-based method and the percentiles of (5), (6), and (7) for the GPI method. The coverage of the REML-based method was far from the nominal level 0.95, especially for low values of the shrinkage factor k. The coverage of the GPI method was closer to the nominal level in all situations.

Estimated coverage of the Gamage et al. (2013) prediction intervals (41) is shown in Table 2. The 0.95 GPIs for $\mu_1^* = \mu + k(\bar{y}_{1\cdot} - \mu)$ in model (1) were computed using the percentiles of (41). Coverage was close to 95%, in agreement with the results of Gamage et al. (2013). However, it was noted that these intervals should not be used as prediction intervals for $\mu + u_1$, since as such, coverage is substantially lower than the nominal level.

In Table 3, results are provided for the one-way unbalanced layout discussed in Section 2.2. Coverage was obtained for prediction intervals for $\mu + u_1$, u_1, and $u_1 - u_2$, computed using (40) for the REML-based and (8), (9), and (10) for the GPI method. The estimated coverage was closer to 0.95 using the GPI method than using the REML-based method.

For the two-way random-effects model (11), Table 4 presents estimated coverage for the REML-based and GPI methods. Results are presented for both balanced and unbalanced layouts. For the REML-based prediction intervals, the percentiles of (40) were used. Using the GPI method, the percentiles of (16), (17), (18), (23), (24), and (25) were used. In all cases, the estimated coverage was closer to 0.95 using the GPI method than using the REML-based method.

Table 5 presents estimated coverage of prediction intervals for $\mu + u_1$, u_1, and $u_1 - u_2$ in two-way random-effects models (26) and (35), which include effects of interaction. For the REML-based method, the percentiles of (40) were applied for both models. Using the GPI method, prediction intervals were computed using the percentiles of (32), (33), and (34) for model (26),

TABLE 6 Estimated coverage of approximate 0.95 prediction intervals for $\mu + u_1$, u_1 and $u_1 - u_2$ in the two-way model without interaction, balanced data, using t-distributions with degrees of freedom computed with the containment (C), the Satterthwaite (SAT) and the Kenward and Roger (KR) methods

		$\mu + u_1$			u_1			$u_1 - u_2$		
k	σ_A^2	C	SAT	KR	C	SAT	KR	C	SAT	KR
0	0	0.972	0.961	0.967	0.460	0.461	0.463	0.456	0.458	0.462
0.25	2	0.969	0.954	0.962	0.604	0.649	0.654	0.599	0.644	0.650
0.40	4	0.969	0.947	0.961	0.704	0.773	0.780	0.702	0.766	0.776
0.50	6	0.974	0.948	0.962	0.773	0.844	0.855	0.767	0.836	0.851
0.60	9	0.976	0.940	0.957	0.833	0.895	0.909	0.827	0.883	0.903
0.70	14	0.984	0.942	0.954	0.881	0.927	0.942	0.881	0.915	0.937
0.75	18	0.987	0.944	0.957	0.904	0.939	0.955	0.901	0.924	0.945
0.80	24	0.988	0.940	0.953	0.915	0.938	0.954	0.921	0.935	0.953
0.85	34	0.992	0.947	0.956	0.932	0.945	0.956	0.931	0.937	0.953
0.90	54	0.993	0.945	0.951	0.935	0.942	0.949	0.942	0.941	0.951

TABLE 7 Observed yield (dt/ha) in an agricultural field experiment with ten varieties of oats (columns) grown in four blocks (rows)

	a_1	a_2	a_3	a_4	a_5	a_6	a_7	a_8	a_9	a_{10}
b_1	55.65	51.82	51.86	66.49	72.41	80.86	54.18	51.47	72.57	68.11
b_2	67.65	73.79	66.19	72.53	66.61	74.46	60.05	59.69	73.27	71.41
b_3	64.90	71.22	61.56	66.03	62.78	78.40	58.22	64.97	73.66	69.73
b_4	78.67	69.16	76.84	74.34	75.41	74.65	60.98	63.91	81.20	74.00

and using the percentiles of (36), (37), and (38) for model (35). In most cases, estimated coverage was closer to the nominal level using the GPI method than using the REML-based method.

For the results of Table 6, model (11) was adopted. The containment, the Satterthwaite (1946) and the Kenward and Roger (1997) methods, as implemented in the `SAS` software package, were applied for calculation of approximate prediction intervals. In almost all cases, coverage was lower than 0.95. For small values of k, that is, when shrinkage was large, coverage was much lower than 0.95. The results of Table 6 can be compared with the results of the upper part of Table 4, which also refers to model (11) used on balanced datasets.

Especially for u_1 and $u_1 - u_2$, the GPI intervals outperformed the containment, Satterthwaite (1946) and Kenward and Roger (1997) intervals with regard to coverage. Unexpectedly, for u_1 and $u_1 - u_2$, the coverage of the containment, the Satterthwaite (1946) and the Kenward and Roger (1997) methods were lower than that of the REML method when $\sigma_A^2 = 0$. The reason for this is that the REML procedure as implemented in the mixed procedure of `SAS` estimates σ_A^2 to zero more often than the REML procedure as implemented in the `lmer` function of `R`. When the estimate of σ_A^2 approaches zero, then the length of the prediction interval approaches zero. In cases when the estimate of σ_A^2 was zero, we defined coverage to be zero. Since SAS more often than R estimated σ_A^2 to zero in the case $\sigma_A^2 = 0$, coverage was smaller using SAS than using R in that specific case.

5 | EXAMPLE

Table 7, which is classified by varieties of oats (columns) and blocks (rows), includes data from an agricultural field experiment. Ten varieties of oats were randomly allocated to plots within four blocks. The observations are yields (dt/ha).

Model (11) was adopted. The estimated overall mean was $\bar{y}_{..} = 67.792$, and the variance component estimates were $\hat{\sigma}_A^2 = 29.091$, $\hat{\sigma}_b^2 = 15.577$ and $\hat{\sigma}_e^2 = 26.999$. Generating 10,000 random samples of $G(\sigma_A^2)$, $G(\sigma_b^2)$, $G(\sigma_e^2)$ and $G(\mu)$, GPIs were computed using the 2.5th and 97.5th percentiles of (16), (17) and (18). The 0.95 GPIs for $\mu + u_1$, u_1, and $u_1 - u_2$ were (61.059,73.000), $(-6.881, 5.002)$, and $(-6.575, 6.720)$, respectively.

Figure 1 shows confidence curves (Schweder & Hjort, 2016) for $\mu + u_1$, u_1 and $u_1 - u_2$, in which the GPIs are displayed for arbitrary confidence levels, together with boxplots that illustrate the empirical conditional distributions.

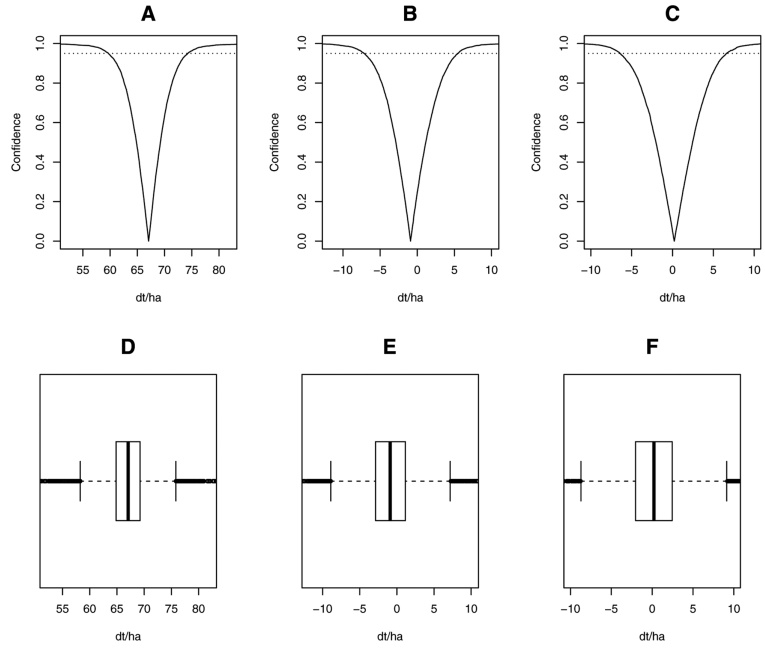

FIGURE 1 Confidence curves for (A) $\mu + u_1$, (B) u_1 and (C) $u_1 - u_2$. Boxplots of 10,000 random observations of (D) $\mu + u_1$, (E) u_1, and (F) $u_1 - u_2$

6 | DISCUSSION

This article considered random-effects models for analysis of comparative experiments, since such models may reduce the MSEs in the estimates of the treatment differences. Our main interest was to propose prediction intervals for differences between experimental treatments when random-effects models are used.

An important finding of the current study is that the standard method for computing prediction intervals for random effects, that is, the REML-based method, often yields too low coverage, deviating substantially from the nominal confidence level. This also holds for the approximate t-distribution methods of Satterthwaite (1946) and Kenward and Roger (1997). The low coverage observed for these methods can be attributed to their property that the lengths of the intervals diminish toward zero as the estimate, $\hat{k}$, of the shrinkage factor approaches zero, which is the case when $\hat{\sigma}_A^2$ becomes small as compared to $\hat{\sigma}_e^2$. In the extreme, when $\hat{k} = 0$, the intervals degenerate. In small experiments, this scenario is not unlikely (Forkman & Piepho, 2013). It is well known that the Kenward and Roger (1997) method works well for fixed effects (Spilke, Piepho, & Hu, 2005). According to our study, the Kenward and Roger (1997) method does not to work well for random effects, which has not been much noted previously.

Gamage et al. (2013) suggested a method for computation of a prediction interval for the predictor $\mu + k(\bar{y}_{1\cdot} - \mu)$ of the treatment mean $\mu + u_1$. Our simulation study confirmed their results on the performance of this prediction interval. The present article proposed prediction intervals for $\mu + u_1$, u_1, and $u_1 - u_2$.

Comparative trials aim at estimating differences between experimental treatments (Bailey, 2008). It is a well-known problem that experiments with many treatments give rise to many pairwise comparisons. The length of the confidence intervals can be adjusted for multiple comparisons by using the nominal level $1 - \alpha/p$, where p is the number of confidence intervals, instead of $1 - \alpha$ (Wellek, 2017).

GCIs are approximate by nature, but asymptotically correct under specific conditions (Hannig et al., 2006). In unbalanced settings, our proposed GPIs are additionally imprecise due to nonindependent approximate chi-square distributions that are not free of parameters, and conditioning on certain averages. The question on how to deal with negative GPQs for variance components is challenging. Despite all approximation, coverage using the proposed GPI methods is still better than coverage using the

REML-based method. The main idea of the proposed GPI methods is that prediction intervals are calculated via simulation of conditional distributions that are dependent on unknown parameters whose uncertainties are included in the intervals through replacement of the variance components by their respective GPQs.

In our proposed procedures, the random components of the GPQs are always sampled independently of each other. In unbalanced cases performance could perhaps be improved by sampling GPQs nonindependently.

In summary, we have developed the GPI method for calculating prediction intervals for linear combinations of random effects. The GPI method showed better coverage than the REML-based procedures. The method can be applied to more complicated models than was detailed here.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ORCID

Razaw Al-Sarraj http://orcid.org/0000-0002-2190-3650
Johannes Forkman http://orcid.org/0000-0002-5796-0710

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SUPPORTING INFORMATION

Additional Supporting Information including source code to reproduce the results may be found online in the supporting information tab for this article.

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APPENDIX A

A.1 Joint distributions

(i) One-way random-effects model, balanced data

$$\begin{pmatrix} \mu + u_1 \\ \bar{y}_{1.} \end{pmatrix} \sim N\left(\begin{pmatrix} \mu \\ \mu \end{pmatrix}, \begin{pmatrix} \sigma_A^2 & \sigma_A^2 \\ \sigma_A^2 & \sigma_A^2 + \frac{\sigma_e^2}{n} \end{pmatrix} \right)$$

$$\begin{pmatrix} u_1 \\ \bar{y}_{1.} - \bar{y}_{..} \end{pmatrix} \sim N\left(\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_A^2 & \sigma_A^2 \left(\frac{a-1}{a} \right) \\ \sigma_A^2 \left(\frac{a-1}{a} \right) & \left(\sigma_A^2 + \frac{\sigma_e^2}{n} \right) \left(\frac{a-1}{a} \right) \end{pmatrix} \right)$$

$$\begin{pmatrix} u_1 - u_2 \\ \bar{y}_{1.} - \bar{y}_{2.} \end{pmatrix} \sim N\left(\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} 2\sigma_A^2 & 2\sigma_A^2 \\ 2\sigma_A^2 & 2\left(\sigma_A^2 + \frac{\sigma_e^2}{n} \right) \end{pmatrix} \right)$$

(ii) One-way random-effects model, unbalanced data

$$\begin{pmatrix} \mu + u_1 \\ \bar{y}_{1.} \end{pmatrix} \sim N\left(\begin{pmatrix} \mu \\ \mu \end{pmatrix}, \begin{pmatrix} \sigma_A^2 & \sigma_A^2 \\ \sigma_A^2 & \sigma_A^2 + \frac{\sigma_e^2}{n_1} \end{pmatrix} \right)$$

$$\begin{pmatrix} u_1 \\ \bar{y}_{1.} - \bar{y}_{..} \end{pmatrix} \sim N\left(\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_A^2 & \sigma_A^2\left(\frac{a-1}{a}\right) \\ \sigma_A^2\left(\frac{a-1}{a}\right) & \sigma_A^2\left(\frac{a-1}{a}\right) + \sigma_e^2\left(\frac{1}{a\tilde{n}_h} + \frac{1}{n_1} - \frac{2}{an_1}\right) \end{pmatrix} \right)$$

$$\begin{pmatrix} u_1 - u_2 \\ \bar{y}_{1.} - \bar{y}_{2.} \end{pmatrix} \sim N\left(\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} 2\sigma_A^2 & 2\sigma_A^2 \\ 2\sigma_A^2 & 2\sigma_A^2 + \sigma_e^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right) \end{pmatrix} \right)$$

(iii) Two-way random-effects model without interaction, balanced data

$$\begin{pmatrix} \mu + u_1 \\ \bar{y}_{1.} \end{pmatrix} \sim N\left(\begin{pmatrix} \mu \\ \mu \end{pmatrix}, \begin{pmatrix} \sigma_A^2 & \sigma_A^2 \\ \sigma_A^2 & \sigma_A^2 + \frac{1}{b}\left(\sigma_b^2 + \sigma_e^2\right) \end{pmatrix} \right)$$

$$\begin{pmatrix} u_1 \\ \bar{y}_{1.} - \bar{y}_{..} \end{pmatrix} \sim N\left(\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_A^2 & \sigma_A^2\left(\frac{a-1}{a}\right) \\ \sigma_A^2\left(\frac{a-1}{a}\right) & \left(\sigma_A^2 + \frac{\sigma_e^2}{b}\right)\left(\frac{a-1}{a}\right) \end{pmatrix} \right)$$

$$\begin{pmatrix} u_1 - u_2 \\ \bar{y}_{1.} - \bar{y}_{2.} \end{pmatrix} \sim N\left(\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} 2\sigma_A^2 & 2\sigma_A^2 \\ 2\sigma_A^2 & 2\left(\sigma_A^2 + \frac{\sigma_e^2}{b}\right) \end{pmatrix} \right)$$

(iv) Two-way random-effects model without interaction, unbalanced data

$$\begin{pmatrix} \mu + u_1 \\ \bar{y}_{1.} \end{pmatrix} \sim N\left(\begin{pmatrix} \mu \\ \mu \end{pmatrix}, \begin{pmatrix} \sigma_A^2 & \sigma_A^2 \\ \sigma_A^2 & \sigma_A^2 + \frac{1}{n_1}\left(\sigma_b^2 + \sigma_e^2\right) \end{pmatrix} \right)$$

$$\begin{pmatrix} u_1 \\ \bar{y}_{1.} - \bar{y}_{..} \end{pmatrix} \sim N\left(\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_A^2 & \sigma_A^2\left(\frac{a-1}{a}\right) \\ \sigma_A^2\left(\frac{a-1}{a}\right) & \sigma_A^2\left(\frac{a-1}{a}\right) + \sigma_b^2\left(\frac{1}{n_1} + \frac{\sum_{p=1}^{a}\sum_{q=1}^{a} d_{pq}}{a^2} + \right. \right. \\ & \left. \left. -\frac{2}{an_1} - \frac{2\sum_{q=2}^{a} d_{1q}}{a}\right) + \sigma_e^2\left(\frac{1}{n_1} + \frac{1}{a\tilde{n}_h} - \frac{2}{an_1}\right) \right) \end{pmatrix} \right)$$

$$\begin{pmatrix} u_1 - u_2 \\ \bar{y}_{1.} - \bar{y}_{2.} \end{pmatrix} \sim N\left(\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} 2\sigma_A^2 & 2\sigma_A^2 \\ 2\sigma_A^2 & 2\sigma_A^2 + \sigma_b^2\left(\frac{1}{n_1} + \frac{1}{n_2} + \right. \right. \\ & \left. \left. - 2d_{12}\right) + \sigma_e^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right) \end{pmatrix} \right)$$

(v) Two-way random-effects model with interaction, balanced data

$$\begin{pmatrix} \mu + u_1 \\ \bar{y}_{1..} \end{pmatrix} \sim N\left(\begin{pmatrix} \mu \\ \mu \end{pmatrix}, \begin{pmatrix} \sigma_A^2 & \sigma_A^2 \\ \sigma_A^2 & \sigma_A^2 + \frac{1}{b}\left(\sigma_b^2 + \sigma_{ab}^2 + \frac{\sigma_e^2}{n}\right) \end{pmatrix} \right)$$

$$\begin{pmatrix} u_1 \\ \bar{y}_{1..} - \bar{y}_{...} \end{pmatrix} \sim N\left(\begin{pmatrix}0\\0\end{pmatrix}, \begin{pmatrix} \sigma_A^2 & \sigma_A^2\left(\frac{a-1}{a}\right) \\ \sigma_A^2\left(\frac{a-1}{a}\right) & \left(\sigma_A^2 + \frac{\sigma_{ab}^2}{b} + \frac{\sigma_e^2}{bn}\right)\left(\frac{a-1}{a}\right)\end{pmatrix}\right)$$

$$\begin{pmatrix} u_1 - u_2 \\ \bar{y}_{1..} - \bar{y}_{2..} \end{pmatrix} \sim N\left(\begin{pmatrix}0\\0\end{pmatrix}, \begin{pmatrix} 2\sigma_A^2 & 2\sigma_A^2 \\ 2\sigma_A^2 & 2\left(\sigma_A^2 + \frac{\sigma_{ab}^2}{b} + \frac{\sigma_e^2}{bn}\right)\end{pmatrix}\right)$$

(vi) Two-way random-effects model with interaction, unbalanced data

$$\begin{pmatrix} \mu + u_1 \\ \bar{y}_{1..} \end{pmatrix} \sim N\left(\begin{pmatrix}\mu\\\mu\end{pmatrix}, \begin{pmatrix} \sigma_A^2 & \sigma_A^2 \\ \sigma_A^2 & \sigma_A^2 + \frac{1}{b}\left(\sigma_b^2 + \sigma_{ab}^2 + \frac{\sigma_e^2}{\tilde{n}_{1h}}\right)\end{pmatrix}\right)$$

$$\begin{pmatrix} u_1 \\ \bar{y}_{1..} - \bar{y}_{...}) \end{pmatrix} \sim N\left(\begin{pmatrix}0\\0\end{pmatrix}, \begin{pmatrix} \sigma_A^2 & \sigma_A^2\left(\frac{a-1}{a}\right) \\ \sigma_A^2\left(\frac{a-1}{a}\right) & \left(\sigma_A^2 + \frac{\sigma_{ab}^2}{b}\right)\left(\frac{a-1}{a}\right) + \frac{\sigma_e^2}{b}\left(\frac{1}{\tilde{n}_{1h}} - \frac{1}{a\tilde{n}_h}\right)\end{pmatrix}\right)$$

$$\begin{pmatrix} u_1 - u_2 \\ \bar{y}_{1..} - \bar{y}_{2..} \end{pmatrix} \sim N\left(\begin{pmatrix}0\\0\end{pmatrix}, \begin{pmatrix} 2\sigma_A^2 & 2\sigma_A^2 \\ 2\sigma_A^2 & 2\sigma_A^2 + \frac{2\sigma_{ab}^2}{b} + \frac{\sigma_e^2}{b}\left(\frac{1}{\tilde{n}_{1h}} + \frac{1}{\tilde{n}_{2h}}\right)\end{pmatrix}\right)$$

A.2 Conditional distribution

Let $\mathbf{Y} \sim N(\mu, \Sigma)$. Partition $\mathbf{Y}$, μ, and Σ as follows:

$$Y = \begin{pmatrix} Y_1 \\ Y_2 \end{pmatrix}, \quad \mu = \begin{pmatrix} \mu_1 \\ \mu_2 \end{pmatrix} \quad \text{and} \quad \Sigma = \begin{pmatrix} \Sigma_{11} & \Sigma_{12} \\ \Sigma_{21} & \Sigma_{22} \end{pmatrix}.$$

The joint distribution can be written as:

$$\begin{pmatrix} Y_1 \\ Y_2 \end{pmatrix} \sim N\left(\begin{pmatrix} \mu_1 \\ \mu_2 \end{pmatrix}, \begin{pmatrix} \Sigma_{11} & \Sigma_{12} \\ \Sigma_{21} & \Sigma_{22} \end{pmatrix}\right).$$

Conditional on $Y_2 = a$, the distribution is normal with expectation and variance (Mardia, Kent, & Bibby, 1979):

$$\mathrm{E}(Y_1|Y_2 = a) = \mu_1 + \Sigma_{12}\Sigma_{22}^{-1}(a - \mu_2),$$

and

$$\mathrm{Var}(Y_1|Y_2 = a) = \Sigma_{11} - \Sigma_{12}\Sigma_{22}^{-1}\Sigma_{21}.$$

A.3 Shrinkage factors

$$\hat{k}_{11} = G(\sigma_A^2)/\left(G(\sigma_A^2) + G(\sigma_e^2)/n\right).$$
$$\hat{k}_{21} = G(\sigma_A^2)/\left(G(\sigma_A^2) + G(\sigma_e^2)/n_1\right).$$
$$\hat{k}_{22} = G(\sigma_A^2)(1 - 1/a)/\left(G(\sigma_A^2)(1 - 1/a) + G(\sigma_e^2)(1/(a\tilde{n}_h) + 1/n_1 - 2/(an_1))\right).$$

$$\hat{k}_{23} = 2G(\sigma_A^2)/\left(2G(\sigma_A^2) + G(\sigma_e^2)(1/n_1 + 1/n_2)\right).$$

$$\hat{k}_{31} = G(\sigma_A^2)/\left(G(\sigma_A^2) + \left(G(\sigma_b^2 + G(\sigma_e^2)\right)/b\right).$$

$$\hat{k}_{32} = G(\sigma_A^2)/\left(G(\sigma_A^2) + G(\sigma_e^2)/b\right).$$

$$\hat{k}_{41} = G(\sigma_A^2)/\left(G(\sigma_A^2) + (G(\sigma_b^2) + G(\sigma_e^2))/n_1\right).$$

$$\hat{k}_{42} = (G(\sigma_A^2)(a-1)/a)/\left(\left(G(\sigma_A^2)(a-1)/a\right) + G(\sigma_b^2)\left(1/n_1 + \sum_{p=1}^{a}\sum_{q=1}^{a} d_{pq}/a^2 - 2/an_1 + \right.\right.$$
$$\left.\left. -2\sum_{q=2}^{a} d_{1q}/a) + G(\sigma_e^2)(1/n_1 + 1/a\tilde{n}_h - 2/an_1)\right)\right).$$

$$\hat{k}_{43} = 2G(\sigma_A^2)/\left(2G(\sigma_A^2) + G(\sigma_b^2)(1/n_1 + 1/n_2 - 2d_{12}) + G(\sigma_e^2)(1/n_1 + 1/n_2)\right).$$

$$\hat{k}_{51} = G(\sigma_A^2)/\left(G(\sigma_A^2) + G(\sigma_b^2)/b + G(\sigma_{ab}^2)/b + G(\sigma_e^2)/(bn)\right)\right).$$

$$\hat{k}_{52} = G(\sigma_A^2)/\left(G(\sigma_A^2) + G(\sigma_{ab}^2)/b + G(\sigma_e^2)/(bn)\right).$$

$$\hat{k}_{61} = G(\sigma_A^2)/\left(G(\sigma_A^2) + G(\sigma_b^2)/b + G(\sigma_{ab}^2)/b + G(\sigma_e^2)/(b\tilde{n}_{1h})\right).$$

$$\hat{k}_{62} = G(\sigma_A^2)(1-1/a)/\left(G(\sigma_A^2) + G(\sigma_{ab}^2)/b\right)(1-1/a) + G(\sigma_e^2)(1/(b\tilde{n}_{1h}) - 1/(ab\tilde{n}_h)).$$

$$\hat{k}_{63} = 2G(\sigma_A^2)/\left(2G(\sigma_A^2) + 2G(\sigma_{ab}^2)/b + G(\sigma_e^2)(1/(b\tilde{n}_{1h}) + 1/(b\tilde{n}_{2h}))\right).$$

A.4 The computation of m_a and m_b

In model (39), let $X_1 = [\mathbf{X}|Z]$ and $Q = L^\top(L(X_1^\top X_1)^- L^\top)^{-1}L$, where L is any matrix of full row rank in the row space of X_1 and $(X_1^\top X_1)^-$ is any generalized inverse of $X_1^\top X_1$. The values m_a and m_b are calculated as $m_a = \text{trace}(Q)/(a-1)$ and $m_b = \text{trace}(Q)/(b-1)$ (Goodnight & Speed, 1978).

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This thesis investigates alternatives to likelihood-based procedures for analysis of factorial experiments with normally distributed observations. Henderson's method 3 for variance component estimation is modified and improved with regard to mean square error. The modified estimator is tested on QTL data. Prediction intervals are derived based on generalized inference methodology. With focus on Type I error, the implications of the available options in SAS and R are examined for the inference on the fixed effects.

Razaw Al-Sarraj received her postgraduate education at the Department of Energy and Technology, SLU, Uppsala, Sweden. She holds a Bachelor of Science in Statistics from University of Mustansiriya, Iraq and a Master of Science in Statistics from University of Salahaddin, Iraq.

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