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## REGULAR ARTICLE

# A two-stage approach to recovery of inter-block information and shrinkage of block effect estimates 

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#### Abstract

Linear mixed models often comprise several effects, and the focus is usually only on one or a few of them, while the other effects need to be fitted merely to adjust for all sources of variation. A typical example is the analysis of a blocked experiment, where the effects of interest pertain to treatments, while effects for replicates and incomplete blocks need to be taken into account in order to obtain efficient estimates of treatment effects. If fixed and random effects that are not of major interest were known beforehand, we could subtract these from the observed data, and a reduced model could then be fitted to these corrected data in order to estimate the effects of interest. In practice, this approach cannot be used directly, however, because true values of the effects are unknown. But we may replace unknown effects by their estimates. We show in this paper, that a reduced model fitted to these 'empirically' corrected data yields BLUE and BLUP of fixed and random effects of interest in the full model. Using examples, this result is demonstrated to be useful for illustrating the recovery of inter-block information and for understanding the properties of estimators obtained from mixed-model analysis.


Key Words: intra-block analysis; linear model; linear mixed model; recovery of inter-block information; shrinkage; sweeping.

## INTRODUCTION

Linear models can be fitted to data in order to make inferences for effects of interest, while controlling for other (nuisance) effects that are not themselves of primary interest, but are important sources of variation. For example, analysis of data from a blocked experiment can be based on the linear model

$$
\begin{equation*}
y_{i j}=\mu_{i}+b_{j}+e_{i j} \tag{1}
\end{equation*}
$$

where $y_{i j}$ is the response of the $i$-th treatment $(i=1, \ldots, I)$ in the $j$-th block $(j=1, \ldots, J)$, $\mu_{i}$ is the mean of the $i$-th treatment, $b_{j}$ is the fixed effect of the $j$-th block, subject to the constraint $\sum_{j=1}^{J} b_{j}=0$, and $e_{i j}$ is the random error term associated with $y_{i j}$, assumed to be independently normally distributed with constant variance $\sigma_{e}^{2}$. We are interested in making inferences regarding the treatment means, while block effects need to be accounted for to represent the randomization layout and to increase precision.

Analysis based on model (1) entails estimating all effects simultaneously and then deriving inference for effects of interest. In our experience from teaching linear model concepts, however, many researchers prefer a two-stage view, according to which the data are first corrected for nuisance effects, and the corrected data, now presumed to be free of nuisance effects, are then subjected to analysis focusing on the effects of interest. In the example, if we knew the values of block effects, we could subtract these from both sides of (1) and then perform an analysis based on the resulting reduced model, which in this case would boil down to a one-way analysis of variance. In practice, the nuisance effects are not known but have to be estimated. The purpose of this paper is to show that an analysis of the corrected data using a model reduced by first accounting for estimated nuisance effects yields the best point estimates of fixed and random effects of interest, i.e., the best linear unbiased estimators (BLUE) and best linear unbiased predictors (BLUP), respectively. The method does not usually provide any computational advantages over the standard procedures, but we have found it to be useful for teaching purposes because of its intuitive appeal and interpretive insights. In particular, the method can be used to explain the socalled recovery of inter-block information (Yates, 1940) in blocked experiments. In this paper, we first develop the general result in Section 2. This is then applied to special cases in Section 3.

## The linear mixed model

Consider the linear mixed model (McLean et al., 1991; Searle et al., 1992)

$$
\begin{equation*}
y=X \beta+Z u+e \tag{2}
\end{equation*}
$$

where $X$ and $Z$ are known design matrices for fixed effects $\beta$ and random effects $u$, respectively. The expected values of random effects are $E(u)=0$ and $E(e)=0$, the variances are $\operatorname{var}(u)=G$ and $\operatorname{var}(e)=\sigma_{e}^{2} I$, and the covariance is $\operatorname{cov}(u, e)=0$, so that $\operatorname{var}(y)=V=Z G Z^{T}+\sigma_{e}^{2} I$. It is assumed here that the positive-definite variance-covariance matrix $G$ and the residual variance $\sigma_{e}^{2}$ are known. We further assume that model (2) can be partitioned as follows:

$$
\begin{align*}
& X \beta=X_{1} \beta_{1}+X_{2} \beta_{2} \text { and }  \tag{3}\\
& Z u=Z_{1} u_{1}+Z_{2} u_{2} \tag{4}
\end{align*}
$$

where $\operatorname{var}\left(u_{1}\right)=G_{1}, \operatorname{var}\left(u_{2}\right)=G_{2}$ and $\operatorname{cov}\left(u_{1}, u_{2}\right)=0$. We assume here that both $X_{1}$ and $X_{2}$ have full column rank and that $s p(X)=s p\left(X_{1}\right) \cup s p\left(X_{2}\right)$, where $s p(A)$ denotes the column space generated by a matrix $A$. The effects of principal interest are taken to be $\beta_{1}$ and $u_{1}$, but the analysis also needs to account for the other effects $\beta_{2}$ and $u_{2}$, which are
themselves not of intrinsic interest. The solution of the mixed model equations pertaining to $\beta$ is

$$
\begin{equation*}
\hat{\beta}=\left(X^{T} V^{-1} X\right)^{-1} X^{T} V^{-1} y=\binom{\hat{\beta}_{1}}{\hat{\beta}_{2}}=\binom{C_{11} X_{1}^{T}+C_{12} X_{2}^{T}}{C_{21} X_{1}^{T}+C_{22} X_{2}^{T}} V^{-1} y \tag{5}
\end{equation*}
$$

where we have used $\left(X^{T} V^{-1} X\right)^{-1}=\left(\begin{array}{ll}X_{1}^{T} V^{-1} X_{1} & X_{1}^{T} V^{-1} X_{2} \\ X_{2}^{T} V^{-1} X_{1} & X_{2}^{T} V^{-1} X_{2}\end{array}\right)^{-1}=\left(\begin{array}{ll}C_{11} & C_{12} \\ C_{21} & C_{22}\end{array}\right)$ with $A^{-1}$ denoting the inverse of $A$. The best linear unbiased estimator (BLUE) of estimable functions $K \beta$ is given by $K \hat{\beta}$ (McLean et al., 1991). The best linear unbiased predictor (BLUP) of $u$ is

$$
\begin{equation*}
\hat{u}=G Z^{T} V^{-1}(y-X \hat{\beta})=\binom{\hat{u}_{1}}{\hat{u}_{2}}=\binom{G_{1} Z_{1}^{T}}{G_{2} Z_{2}^{T}} V^{-1}(y-X \hat{\beta}) \tag{6}
\end{equation*}
$$

We now consider a two-stage representation of the BLUE and BLUP in (5) and (6), respectively, in which we correct the observed data for nuisance effects $\beta_{2}$ and $u_{2}$ in the first stage. In the second stage the corrected data are used to estimate $\beta_{1}$ and $u_{1}$. The intuition underlying our approach is that if we knew $\beta_{2}$ and $u_{2}$, we could compute the corrected data

$$
\begin{equation*}
y_{c}^{*}=y-X_{2} \beta_{2}-Z_{2} u_{2} \tag{7}
\end{equation*}
$$

and fit the reduced model

$$
\begin{equation*}
y_{c}^{*}=X_{1} \beta_{1}+Z_{1} u_{1}+e \tag{8}
\end{equation*}
$$

with $\operatorname{var}\left(y_{c}^{*}\right)=V_{c}=Z_{1} G_{1} Z_{1}^{T}+\sigma_{e}^{2} I$. In practice, we need to replace the unknown effects $\beta_{2}$ and $u_{2}$ with their estimators, so the corrected data are computed as

$$
\begin{equation*}
y_{c}=y-X_{2} \hat{\beta}_{2}-Z_{2} \hat{u}_{2} \tag{9}
\end{equation*}
$$

where the estimates $\hat{\beta}_{2}$ and $\hat{u}_{2}$ are obtained from (5) and (6), respectively.
Proposition 1: One may analyse $y_{c}$ in (9) as if its variance-covariance structure were the same as that of $y_{c}^{*}$ in (8) under the reduced model. Thus, we may fit the model

$$
\begin{equation*}
y_{c}=X_{1} \beta_{1}+Z_{1} u_{1}+e_{c}, \tag{10}
\end{equation*}
$$

where $e_{c}$ is an error term associated with $y_{c}$, as if $e_{c}=e$. The estimators of $\beta_{1}$ and $u_{1}$ under this naïve model yield the BLUE of $\beta_{1}$ and the BLUP of $u_{1}$ under the full model (1), i.e.,

$$
\begin{equation*}
\widetilde{\beta}_{1}=\left(X_{1}^{T} V_{c}^{-1} X_{1}\right)^{-1} X_{1}^{T} V_{c}^{-1} y_{c}=\hat{\beta}_{1} \text { and } \tag{11}
\end{equation*}
$$

$$
\begin{equation*}
\tilde{u}_{1}=G_{1} Z_{1}^{T} V_{c}^{-1}\left(y_{c}-X_{1} \tilde{\beta}_{1}\right)=\hat{u}_{1} . \tag{12}
\end{equation*}
$$

Thus, the single-stage estimators of $\beta_{1}$ and $u_{1}$ can be represented in two stages, i.e., we first compute corrected data $y_{c}$, removing the estimates of the nuisance effects $\beta_{2}$ and $u_{2}$, and then use the estimators in (11) and (12). A proof of Proposition 1 is given in the Appendix.

We note here that while equations (11) and (12) yield correct point estimates of effects, they do not lend themselves for further statistical inference (significance tests or confidence intervals). For example, the variance of $\tilde{\beta}_{1}$ is certainly not generally equal to $\left(X_{1}^{T} V_{c}^{-1} X_{1}\right)^{-1}$. Also, a valid estimate of $V_{c}$, i.e., an estimate that is equivalent to the restricted maximum log-likelihood (REML) estimate based on the full model (2), cannot be obtained in the second stage using standard procedures, because of the approximate nature of the error model. Thus, an estimate of $V_{c}$ must be available from the first stage. Moreover, in order to remove $\beta_{2}$ and $u_{2}$, we estimate the full model, which already yields direct estimates of $\beta_{1}$ and $u_{1}$, so our method does not provide any computational advantages. Thus, we do not suggest using this two-stage scheme in practice. The important point to note here is that we may consider this two-stage scheme not only because it leads to the known best estimators, but also because it provides some useful insights into the properties of treatment effect estimators in blocked experiments that are not readily apparent from other methods. This will be illustrated in the following examples.

## Special cases and Examples

## RESULT APPLIED TO A FIXED EFFECTS MODEL

Consider the fixed effects model

$$
\begin{equation*}
y=X_{1} \beta_{1}+X_{2} \beta_{2}+e, \tag{13}
\end{equation*}
$$

where the notation follows that used in (1). For example, $\beta_{1}$ may comprise fixed treatment effects and $\beta_{2}$ may comprise fixed block effects in a randomized block experiment (see eq. 1). We now use corrected data

$$
\begin{equation*}
y_{c}=y-X_{2} \hat{\beta}_{2}, \tag{14}
\end{equation*}
$$

where $\hat{\beta}_{2}$ is the ordinary least squares estimator of $\beta_{2}$ under model (13). Of course, the distributional properties of $y_{c}$ in (14) are not identical to those of $y-X_{2} \beta_{2}$, but from Proposition 1, an analysis that naïvely pretends that they are, does, in fact, yield the BLUE of $\beta_{1}$. Hence, using the corrected data $y_{c}$ in (14), we fit the linear model

$$
\begin{equation*}
y_{c}=X_{1} \beta_{1}+e_{c} \tag{15}
\end{equation*}
$$

by ordinary least squares, yielding the estimator

$$
\begin{equation*}
\widetilde{\beta}_{1}=\left(X_{1}^{T} X_{1}\right)^{-1} X_{1}^{T} y_{c} . \tag{16}
\end{equation*}
$$

It follows from Proposition 1 that the two-stage estimator in (16) is equivalent to the BLUE of $\beta_{1}$ based on (13).

Table 1: Yoghurt tasting raw data (Mead et al., 1993, p.81) and data corrected for estimated block effects assuming either fixed or random block effects (see Table 2), $y_{c(i j)}=y_{i j}-\hat{b}_{j}$, and arithmetic treatment means computed from these corrected data. Fixed effects were estimated by BLUE, random effect estimates were obtained by BLUP.

|  |  | Taster |  |  |  |  |
| :---: | :--- | :---: | :---: | :---: | :---: | :---: |
| Yoghurt | Data | 1 | 2 | 3 | 4 | Mean |
| A | Raw data | Corrected - blocks fixed | 6 | 5 | 9 |  |
|  | Corrected - blocks random | 6.625 | 6.750 | 7.500 |  | 6.333 |
|  | Raw data | 6.750 | 6.591 | 7.636 |  | 6.992 |
|  | B | Corrected - blocks fixed | 4.625 | 3.750 |  | 3.125 |
|  | Corrected - blocks random | 4.750 | 3.591 |  | 3.023 | 3.788 |
|  | Raw data | 4 |  | 4 | 3 | 3.667 |
| C | Corrected - blocks fixed | 2.625 |  | 2.500 | 4.125 | 3.083 |
|  | Corrected - blocks random | 2.750 |  | 2.636 | 4.023 | 3.136 |
|  | Raw data |  | 4 | 7 | 4 | 5.000 |
| D | Corrected - blocks fixed |  | 5.750 | 5.500 | 5.125 | 5.458 |
|  | Corrected - blocks random |  | 5.591 | 5.636 | 5.023 | 5.417 |

Example 1: In order to illustrate our result as applied to a fixed-effects model, we consider the yoghurt tasting data described in Mead et al. (1993, p.81). The data is reproduced in Table 1. The experiment was laid out according to a balanced incomplete block design. We use model (1) for analysis, taking tasters as blocks and yoghurts as treatments. Treatment means correspond to $\beta_{1}$, while block effects relate to $\beta_{2}$ in model (13). Least squares estimates for taster effects are given in Table 2. Note that the design matrix $X_{2}$ for fixed block effects has full column rank if we replace the last block effect by $b_{J}=-\sum_{j=1}^{J-1} b_{j}$, in accordance with the constraint $\sum_{j=1}^{J} b_{j}=0$, so that $X_{2}$ has $(J-1)$ linearly independent columns. The observed data were corrected using $y_{c(i)}=y_{i j}-\hat{b}_{j}$ (Table 1). We fitted the reduced model $y_{c(i j)}=\mu_{i}+e_{c(i j)}$ to the corrected data, which boils down to computing the arithmetic means (i.e., simple averages) of the corrected data for each treatment. These arithmetic means coincide with the adjusted treatment means obtained by directly fitting model (1), which confirms the general result in Proposition 1. This example lucidly illustrates, in an intuitive way, the fact that fitting model (1) provides an adjustment for block effects.

Table 2: Estimates of taster effects $b_{j}$ obtained from a linear model package. Fixed effects were estimated by BLUE (subject to the constraint $\sum_{j=1}^{J} b_{j}=0$ ), random effects were estimated by BLUP.

|  | Taster |  |  |  |
| :--- | :---: | :---: | :---: | :---: |
| Effect estimate | 1 | 2 | 3 | 4 |
| BLUE | 1.375 | -1.750 | 1.500 | -1.125 |
| BLUP | 1.250 | -1.591 | 1.364 | -1.023 |

## RESULT APPLIED TO A MIXED EFFECTS MODEL

Now consider the mixed model (2) and assume that we wish to estimate fixed effects $\beta$, correcting for random effects $u$. In analogy to the fixed effects case, we will use corrected data

$$
\begin{equation*}
y_{c}=y-Z \hat{u}, \tag{17}
\end{equation*}
$$

where $\hat{u}$ denotes the BLUP of $u$ based on (2), and fit the following model to the corrected data:

$$
\begin{equation*}
y_{c}=X \beta+e_{c} . \tag{18}
\end{equation*}
$$

This leads to the estimator

$$
\begin{equation*}
\tilde{\beta}=\left(X^{T} X\right)^{-1} X^{T} y_{c} . \tag{19}
\end{equation*}
$$

From Proposition 1 this estimator is equivalent to the BLUE of $\beta$ under the mixed model (2).

Example 1 (continued): This time, we fit blocks (tasters) in model (1) as random effects in order to recover the inter-block information (Patterson and Thompson, 1971). Thus, treatment means pertain to $\beta$, while block effects are collected in $u$ of (2). Note that the fixed-effects constraint $\sum_{j=1}^{J} b_{j}=0$ is now replaced by the assumption $E\left(b_{j}\right)=0$ for the random block effects. The residual maximum likelihood (REML) estimates of the variance components for blocks and error are $\hat{\sigma}_{b}^{2}=2.5667$ and $\hat{\sigma}_{e}^{2}=0.6833$, respectively. We subtract BLUPs of block effects (Table 2) from the observed response, yielding corrected data $y_{c(i j)}=y_{i j}-\hat{b}_{j}$, and then fit a fixed-effects model with effects for treatment means $\left(y_{c(i j)}=\mu_{i}+e_{c(i j)}\right)$ to the corrected data (Table 1). This is equivalent to computing arithmetic means for $y_{c(i j)}$ per treatment and yields treatment means that are identical to BLUE under the mixed model.

It is noteworthy that the adjusted means based on adjustments with random block effects are closer to the arithmetic yoghurt means than are the adjusted means obtained with fixed block effects (Table 1). Our approach nicely illustrates that this observation, which generally holds for analyses of incomplete block designs, can be explained by the shrinkage of block effect estimates under the random block effects model. The fact that this shrinkage must reduce the adjustment compared to the fixed block effects model can be seen directly from the equation for corrected data $\left(y_{c(i j)}\right)$, and it is readily verified by comparing the adjustments in Table 2.

## RESULT APPLIED TO CORRECTION FOR BOTH FIXED AND RANDOM EFFECTS

In Example 1, we considered correction for a fixed or a random nuisance effect, when the effect of interest is fixed. In more complex models, there may be a need to account for both fixed and random effects. For example, in a resolvable row-column design, rows and columns are incomplete blocking units nested within complete replicates. In the analysis, one may want to correct for fixed effects of complete replicates and for random effects of rows and columns nested within complete replicates. Using the same definitions as before, we may write the mixed model as

$$
\begin{equation*}
y=X_{1} \beta_{1}+X_{2} \beta_{2}+Z u+e, \tag{20}
\end{equation*}
$$

where the fixed effects of interest are $\beta_{1}$, while we want to correct for fixed effects $\beta_{2}$ and random effects $u$. Thus, we compute corrected data

$$
\begin{equation*}
y_{c}=y-X_{2} \hat{\beta}_{2}-Z \hat{u}, \tag{21}
\end{equation*}
$$

where $\hat{\beta}_{2}$ and $\hat{u}$ denote the BLUE of $\beta_{2}$ and the BLUP of $u$, respectively, based on (20). We then fit the model

$$
\begin{equation*}
y_{c}=X_{1} \beta_{1}+e_{c} \tag{22}
\end{equation*}
$$

by ordinary least squares.
Example 2: Table 3 reports data from a field experiment to test 35 wheat genotypes using a resolvable row-column design. A complete replicate is subdivided into incomplete rows and columns. Thus, we have incomplete blocks in both rows and columns. We may either take rows and columns as fixed (intra-block analysis) or random (combined intra-block-interblock analysis with recovery of information). We here chose the latter option.

Table 3: Design and grain yield data for 35 genotypes of wheat in two replicates with five rows and seven columns (reproduced from Table 4.16 of Mead (1997, p. 62). § Numbers refer to genotype IDs.

|  | Design by Genotype ID |  |  |  |  |  |  | Yield |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Replicate 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Col-1 | Col-2 | Col-3 | Col-4 | Col-5 | Col-6 | Col-7 | Col-1 | Col-2 | Col-3 | Col-4 | Col-5 | Col-6 | Col-7 |
| Row 1 | 20 | 4 | 33 | 28 | 7 | 12 | 30 | 3.77 | 3.21 | 4.55 | 4.09 | 5.05 | 4.19 | 3.27 |
| Row 2 | 10 | 14 | 16 | 21 | 31 | 6 | 18 | 3.44 | 4.30 | . | 3.86 | 3.26 | 4.30 | 3.72 |
| Row 3 | 22 | 11 | 19 | 26 | 29 | 15 | 23 | 3.49 | 4.20 | 4.77 | 2.56 | 2.87 | 1.93 | 2.26 |
| Row 4 | 24 | 25 | 5 | 32 | 2 | 27 | 8 | 3.62 | 4.52 | 4.23 | 3.76 | 3.61 | 3.62 | 4.01 |
| Row 5 | 17 | 9 | 3 | 34 | 13 | 35 | 1 | 3.81 | 3.75 | 4.81 | 3.69 | 4.61 | 2.68 | 4.15 |
|  | Replicate 2 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Row 1 | 31 | 19 | 25 | 34 | 20 | 8 | 6 | 4.70 | 7.37 | 5.03 | 5.33 | 5.73 | 4.70 | 5.63 |
| Row 2 | 24 | 21 | 12 | 4 | 23 | 13 | 3 | 4.07 | 5.66 | 4.98 | 4.04 | 4.27 | 4.10 | 4.75 |
| Row 3 | 11 | 7 | 26 | 5 | 35 | 10 | 30 | 5.66 | 6.43 | 4.59 | 5.20 | 4.83 | 4.70 | 4.23 |
| Row 4 | 33 | 9 | 17 | 18 | 32 | 15 | 2 | 5.71 | 6.13 | 4.63 | 5.48 | 5.47 | . | 4.16 |
| Row 5 | 1 | 27 | 16 | 29 | 14 | 28 | 22 | 5.22 | 6.16 | 4.20 | 4.66 | 5.54 | 3.81 | 3.60 |

The mixed model used for analysis is

$$
\begin{equation*}
y_{i j h k}=\mu_{i}+\gamma_{j}+r_{j h}+c_{j k}+e_{i j h k} \tag{23}
\end{equation*}
$$

where
$y_{i j h k}=$ yield of $i$-th genotype in $h$-th row and $k$-th column nested within $j$-th complete replicate
$\mu_{i}=$ mean of $i$-th treatment
$\gamma_{j}=$ effect of $j$-th complete replicate, subject to the constraint $\sum_{j} \gamma_{j}=0$
$r_{j h}=$ effect of $h$-th row within $j$-th replicate; $r_{j h} \sim N\left(0, \sigma_{r}^{2}\right)$
$c_{j k}=$ effect of $k$-th column within $j$-th replicate; $c_{j k} \sim N\left(0, \sigma_{c}^{2}\right)$
$e_{i j h k}=$ residual plot error associated with $y_{i j h k} ; e_{i j h k} \sim N\left(0, \sigma_{e}^{2}\right)$
The REML estimates of the variance components are $\hat{\sigma}_{r}^{2}=0.064, \hat{\sigma}_{c}^{2}=0.192$, and $\hat{\sigma}_{e}^{2}=0.090$. The adjusted genotype means obtained by a REML-based analysis using the model (20) are shown in Table 4. The same estimates are obtained by our step-wise approach as follows:
(i) We first fit (23) to obtain the BLUPs of row and column effects (Table 5) and the BLUEs of $\gamma_{j}\left(\hat{\gamma}_{1}=-\hat{\gamma}_{2}=-0.611\right)$.
(ii) Using these, we compute corrected data $y_{c(j h k)}=y_{i j h k}-\hat{\gamma}_{j}-\hat{r}_{j h}-\hat{c}_{j k}$ (Table 6).
(iii) Computing arithmetic means per genotype for these corrected data, corresponding to an ordinary least squares fit of the model $y_{c(j j h k)}=\mu_{i}+e_{c(j j h k)}$, yields the same means as the adjusted means in Table 4.

Table 4: Adjusted genotype means for wheat data in Table 3 based on model (20).

| ID | Mean | ID | Mean | ID | Mean | ID | Mean | ID | Mean |
| ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| 1 | 4.814 | 8 | 4.602 | 15 | 3.216 | 22 | 4.012 | 29 | 3.794 |
| 2 | 3.915 | 9 | 4.351 | 16 | 3.955 | 23 | 3.424 | 30 | 3.953 |
| 3 | 5.098 | 10 | 4.328 | 17 | 4.153 | 24 | 3.888 | 31 | 3.859 |
| 4 | 3.521 | 11 | 4.932 | 18 | 4.564 | 25 | 4.640 | 32 | 4.264 |
| 5 | 4.396 | 12 | 4.946 | 19 | 5.670 | 26 | 3.759 | 33 | 4.914 |
| 6 | 5.410 | 13 | 4.682 | 20 | 4.319 | 27 | 4.670 | 34 | 4.298 |
| 7 | 5.085 | 14 | 4.765 | 21 | 4.594 | 28 | 4.294 | 35 | 3.602 |

Table 5: BLUPs of row and column effects ( $r_{j h}$ and $c_{j k}$ ) based on model (23) using REML.

| No | Replicate 1 |  | Replicate 2 |  |
| :---: | :---: | :---: | :---: | :---: |
|  | Row | Column | Row | Column |
| 1 | 0.164 | 0.089 | 0.168 | -0.019 |
| 2 | -0.113 | 0.151 | -0.305 | 0.786 |
| 3 | -0.291 | 0.137 | 0.156 | -0.197 |
| 4 | 0.148 | -0.035 | 0.149 | 0.198 |
| 5 | 0.093 | 0.205 | -0.169 | 0.441 |
| 6 |  | -0.384 |  | -0.647 |
| 7 |  | -0.163 |  | -0.562 |

Example 2 (continued): Now assume that the genotype effect is modeled as random and we want to estimate genotype means. Thus, in model (23), we replace $\mu_{i}$ with $\mu+g_{i}$, where $\mu$ is a general mean (fixed) and $g_{i}$ is the random effect of the $i$-th genotype (breeding values), which can be modeled to be correlated, either using pedigree information, or using marker data as in genomic selection (Piepho, 2009). In this case, we can make the following associations with respect to the general model (2): $\beta_{1}=$ fixed intercept, $\beta_{2}=$ fixed replicate effect, $u_{1}=$ random genotype effects, $u_{2}=$ random block effects. Corrected data are computed by subtracting estimates of replicate and block effects. In stage two, ordinary least squares is not the preferred method of estimation, because $V_{c} \neq \sigma_{e}^{2} I$. Instead, we now need to use an estimate of $V_{c}$ obtained from the first stage in order to estimate both $\beta_{1}$ and $u_{2}$ as per equations (11) and (12).

Table 6: Corrected data $y_{c(i j h k)}=y_{i j h k}-\hat{\gamma}_{j}-\hat{r}_{j h}-\hat{c}_{j k}$ for wheat data in Table 3. Fixed effects $\left(\gamma_{j}\right)$ were estimated by BLUE (subject to the constraint $\sum_{j=1}^{J} \gamma_{j}=0$ ), random effects $\left(r_{j h}, c_{j k}\right)$ were estimated by BLUP (Table 5).

|  | Design by Genotype ID |  |  |  |  |  |  | Adjusted Yield |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |  | Replicate 1 |  |  |  |  |  |  |
|  | Col-1 Col-2 Col-3 Col-4 Col-5 Col-6 Col-7 |  |  |  |  |  |  | Col-1 | Col-2 | Col-3 | Col-4 | Col-5 | Col-6 | Col-7 |
| Row 1 | 20 | 4 | 33 | 28 | 7 | 12 | 30 | 4.129 | 3.506 | 4.860 | 4.573 | 5.293 | 5.021 | 3.881 |
| Row 2 | 10 | 14 | 16 | 21 | 31 | 6 | 18 | 4.075 | 4.873 | . | 4.619 | 3.779 | 5.408 | 4.607 |
| Row 3 | 22 | 11 | 19 | 26 | 29 | 15 | 23 | 4.304 | 4.951 | 5.535 | 3.497 | 3.567 | 3.216 | 3.325 |
| Row 4 | 24 | 25 | 5 | 32 | 2 | 27 | 8 | 3.994 | 4.832 | 4.556 | 4.258 | 3.868 | 4.467 | 4.636 |
| Row 5 | 17 | 9 | 3 | 34 | 13 | 35 | 1 | 4.240 | 4.117 | 5.191 | 4.244 | 4.923 | 3.582 | 4.831 |
|  |  |  |  |  |  |  |  | Replicate 2 |  |  |  |  |  |  |
| Row 1 | 31 | 19 | 25 | 34 | 20 | 8 | 6 | 3.940 | 5.805 | 4.448 | 4.353 | 4.510 | 4.567 | 5.413 |
| Row 2 | 24 | 21 | 12 | 4 | 23 | 13 | 3 | 3.782 | 4.568 | 4.871 | 3.536 | 3.522 | 4.440 | 5.005 |
| Row 3 | 11 | 7 | 26 | 5 | 35 | 10 | 30 | 4.912 | 4.878 | 4.020 | 4.235 | 3.622 | 4.580 | 4.025 |
| Row 4 | 33 | 9 | 17 | 18 | 32 | 15 | 2 | 4.968 | 4.584 | 4.067 | 4.522 | 4.269 | . | 3.962 |
| Row 5 | 1 | 27 | 16 | 29 | 14 | 28 | 22 | 4.796 | 4.932 | 3.955 | 4.020 | 4.657 | 4.014 | 3.720 |

## DISCUSSION

This paper has shown how mixed model analyses involving fixed and random effects of interest as well as fixed and random nuisance effects that need to be corrected for can be represented in a two-stage form, where an adjustment for the nuisance effects takes place in the first stage, while estimators of the effects of interest are computed in the second stage. This two-stage representation provides a very intuitive insight into the types of adjustment involved in mixed model analysis. We found this representation to be particularly useful for illustrating adjustments in blocked experiments. In Example 1, the adjustments of the raw data when using a mixed model with random blocks are smaller than when fixed block effects are fitted because the BLUPs of block effects $b_{j}(j=1,2,3,4)$ are shrunk towards zero (Table 1). This nicely reflects the recovery of inter-block information effected by taking blocks as random. To further illustrate this key point, it is worth considering the two extreme cases with respect to the value taken by variance component for blocks ( $\sigma_{b}^{2}$ ) relative to that for error $\left(\sigma_{e}^{2}\right)$. For balanced incomplete block designs, the BLUE of a treatment contrast $\tau=\sum_{i} c_{i} \mu_{i} \quad\left(\sum_{i} c_{i}=0\right)$ can be written (Yates, 1940; Mead, 1997)

$$
\begin{equation*}
\hat{\tau}_{\text {comb }}=\left(\frac{E \hat{\tau}_{\text {intra }}}{\sigma_{e}^{2}}+\frac{(1-E) \hat{\tau}_{\text {inter }}}{\sigma_{e}^{2}+k \sigma_{b}^{2}}\right) /\left(\frac{E}{\sigma_{e}^{2}}+\frac{1-E}{\sigma_{e}^{2}+k \sigma_{b}^{2}}\right) \tag{24}
\end{equation*}
$$

where $E$ is the efficiency factor $(0<E<1), k$ is the block size, and $\hat{\tau}_{\text {intra }}$ and $\hat{\tau}_{\text {inter }}$ are the intra-block and inter-block estimators of the treatment contrast. If the block variance is zero ( $\sigma_{b}^{2}=0$ ), then there is no need for any block adjustments and the BLUE of treatment means are equivalent to simple arithmetic means of raw data $y_{i j}$ for a treatment, corresponding to an analysis based on the model without block effect ( $y_{i j}=\mu_{i}+e_{i j}$ ). In this case, both types of information receive weights depending only on the average efficiency factor $\left(\hat{\tau}_{\text {comb }}=E \hat{\tau}_{\text {intra }}+(1-E) \hat{\tau}_{\text {inter }}\right)$. Conversely, when the block variance becomes very large relative to the error variance ( $\sigma_{b}^{2} / \sigma_{e}^{2} \rightarrow \infty$ ), and hence the block adjustment is maximal, the block effect essentially becomes a fixed effect, because the mixed model equations for blocks then coincide with the ordinary least squares equations for the fixed effects model (Searle et al., 1992, p.276), meaning that all information is intra-block information ( $\hat{\tau}_{\text {comb }}=\hat{\tau}_{\text {intra }}$ ). In practice, when error and block variance are of the same order of magnitude, the block adjustment is intermediate, reflecting the optimally weighted combination of inter-block and intra-block information. Our proposed method shows that this intermediate nature of the combined analysis corresponds to shrinkage of the estimates of block effect estimates. While this same insight is hidden in classical equations such as (24), we think that it becomes much more transparent by inspecting our equation for the corrected data.

In this paper we have assumed in all examples that $\operatorname{var}(e)=\sigma_{e}^{2} I$, which leads to ordinary least squares estimates in stage two of our approach in special cases. In some applications, such as analysis of repeated measures or spatial analysis of field trials, the errors associated with the observational unit have non-zero correlations and so deviate from this simple structure. If the residual error has two components, i.e., one that has a spatial or temporal correlation structure, and one that represents 'white noise' or 'nugget' (Piepho et al., 2008), then the latter component can be associated with the error term such that $\operatorname{var}(e)=\sigma_{e}^{2} I$, while the correlated component is assigned to the random (nuisance) effects $u_{2}$ or $u$. With these assignments, the same simplified results can be obtained as in the examples considered in this paper.

There is an important difference between our approach and the usual method of sweeping (De Hoog et al., 1990). For example, in the fixed-effects linear model (13), the method of sweeping computes "corrected data" $y_{d}=M_{2} y$ with $M_{2}=I-X_{2}\left(X_{2}^{T} X_{2}\right)^{-1} X_{2}^{T}$ to take out the fixed effect $\beta_{2}$. Then the reduced model is $E\left(y_{d}\right)=M_{2} X_{1} \beta_{1}$ and $\operatorname{var}\left(y_{d}\right)=M_{2} \sigma^{2}$, from which the reduced normal equations for $\beta_{1}$ are $\left(X_{1}^{T} M_{2}\right) M_{2}^{-}\left(M_{2} X_{1} \beta_{1}\right)=\left(X_{1}^{T} M_{2}\right) M_{2}^{-}\left(M_{2} y\right)$, where $M_{2}^{-}$denotes a generalized inverse of $M_{2}$, which simplifies to $X_{1}^{T} M_{2} X_{1} \beta_{1}=X_{1}^{T} M_{2} y$ (Searle, 1987, p.263). While this also yields the least squares estimator of $\beta_{1}$ under the model (13), the corrected data $y_{d}$ have different expectation than the corrected data $y_{c}$ in (14), for which $E\left(y_{c}\right)=X_{1} \beta_{1}$. The difference arises because in our approach we use the full model, and hence both $X_{1}$ and $X_{2}$ to obtain the least squares estimate of $\beta_{2}$, which is then used to correct the data, whereas the method of sweeping only uses $X_{2}$ to sweep out $\beta_{2}$. While the method of sweeping has several advantages compared to ours, we find it more difficult to use in teaching non-statisticians, mainly because the reduced model for $y_{d}$ looks more complicated than that for $y_{c}$. Also, our method allows corrected data to be handled as if nuisance effects had never been there in
the first place. By contrast, corrected data obtained by sweeping require accounting for the correction.

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## APPENDIX

## Proof of Proposition 1:

$$
\begin{aligned}
y_{c} & =y-X_{2} \hat{\beta}_{2}-Z_{2} \hat{u}_{2} \\
& =y-X_{2}\left(C_{21} X_{1}^{T} V^{-1} y+C_{22} X_{2}^{T} V^{-1} y\right)-Z_{2} G_{2} Z_{2}^{T} V^{-1}(y-X \hat{\beta}) \\
& =\left[I-\left(X_{2} C_{21} X_{1}^{T} V^{-1}+X_{2} C_{22} X_{2}^{T} V^{-1}\right)-\left(V-V_{c}\right) P\right] y \\
& =\left[V P+\left(X_{1} C_{11} X_{1}^{T} V^{-1}+X_{1} C_{12} X_{2}^{T} V^{-1}\right)-V P+V_{c} P\right] y \\
& =X_{1} \hat{\beta}_{1}+V_{c} P y
\end{aligned}
$$

where

$$
\begin{aligned}
P & =V^{-1}-V^{-1} X\left(X^{T} V^{-1} X\right)^{-1} X^{T} V^{-1} \\
& =V^{-1}-V^{-1}\left(X_{1} C_{11} X_{1}^{T}+X_{1} C_{12} X_{2}^{T}+X_{2} C_{21} X_{1}^{T}+X_{2} C_{22} X_{2}^{T}\right) V^{-1} .
\end{aligned}
$$

With this result, we find

$$
\begin{aligned}
X_{1} \tilde{\beta}_{1} & =X_{1}\left(X_{1}^{T} V_{c}^{-1} X_{1}\right)^{-1} X_{1}^{T} V_{c}^{-1} y_{c} \\
& =X_{1}\left(X_{1}^{T} V_{c}^{-1} X_{1}\right)^{-1} X_{1}^{T} V_{c}^{-1}\left(X_{1} \hat{\beta}_{1}+V_{c} P y\right) \\
& =X_{1}\left(X_{1}^{T} V_{c}^{-1} X_{1}\right)^{-1} X_{1}^{T} V_{c}^{-1} X_{1} \hat{\beta}_{1}=X_{1} \hat{\beta}_{1},
\end{aligned}
$$

where we have used $X_{1}^{T} P=0$ and $X_{1}\left(X_{1}^{T} V_{c}^{-1} X_{1}\right)^{-1} X_{1}^{T} V_{c}^{-1} X_{1}=X_{1}$ (c.f. Rao et al., 2008,
p.218). Thus, $\tilde{\beta}_{1}$ and $\hat{\beta}_{1}$ are identical. Moreover,

$$
\begin{aligned}
\tilde{u}_{1} & =G_{1} Z_{1}^{T} V_{c}^{-1}\left(y_{c}-X_{1} \tilde{\beta}_{1}\right) \\
& =G_{1} Z_{1}^{T} V_{c}^{-1}\left(X_{1} \hat{\beta}_{1}+V_{c} P y-X_{1} \hat{\beta}_{1}\right) \\
& =G_{1} Z_{1}^{T} P y \\
& =G_{1} Z_{1}^{T} V^{-1}(y-X \hat{\beta}) \\
& =\hat{u}_{1} .
\end{aligned}
$$

