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Martin-Luther-University Halle-Wittenberg, Agricultural Faculty, Germany

Analysis of Unbalanced Data by Mixed Linear Models Using the MIXED Procedure of the SAS System

J. Spilke, H. P. Piepho, and X. Hu

Authors' addresses: Prof. Dr J. Spilke (corresponding author; e-mail: spilke@landw.uni-halle.de) and Dr X. Hu, Arbeitsgruppe Biometrie und Agrarinformatik, Martin-Luther-Universität Halle-Wittenberg, 6108 Halle, Ludwig-Wucherer-Straße 82–85, 70593 Stuttgart, Germany; Prof. Dr H. P. Piepho, Institut für Pflanzenbau und Grünland, Universität Hohenheim, Hohenheim, Germany

With 10 tables

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Abstract

Unbalanced data are a common problem in plant research based on designed experiments. Such data are often conveniently analysed using linear mixed models. Recent developments in mixed model theory have been implemented in major packages. This paper describes the use of the MIXED procedure of the SAS System for the analysis of designed experiments. Special emphasis is given to the specification of options as depending on the assumed mixed model and on the unbalancedness in the data. In addition, we consider a compact representation of multiple comparisons for unbalanced data (letter display). Two small data sets are used to exemplify the methods.

Key words: analysis-of-variance — Kenward — Roger method — restricted maximum likelihood — SAS PROC MIXED — Satterthwaite method — Wald test

Introduction

For the majority of agricultural experiments and surveys, the data can be regarded as realizations of a normally distributed random variable with an underlying linear model. Quite often, the linear model contains more than one random source of variation and so is of the mixed type. In addition, more often than not, the data are unbalanced in the sense that the number of observations is not constant across factor combinations. Unbalancedness is an inherent feature of several experimental designs, e.g. lattice designs and cross-over designs. Furthermore, balanced designs may become unbalanced, when one or more observations are lost due to unforeseen circumstances.

A practical problem in the analysis of unbalanced data by mixed models is that usually exact statistical tests are not available, and one needs to resort to approximate methods. Several approximations have been implemented in the MIXED procedure of the SAS System. The approximation appropriate in a given situation depends on the design and the mixed model assumed. The purpose of our contribution is, therefore, to describe the relevant options available in MIXED and give hints regarding the appropriate choice of approximation. Use of the options will be exemplified for two different data sets. Mixed models for these two examples are presented and options for the analysis of mixed models based on these two examples are described.

Mixed models for two data sets

This section describes mixed modelling for two simple examples. Example 1 concerns a single-factor randomized complete block experiment on the yields of different potato cultivars. The second example is a two-factor split-plot experiment with oilseed rape, which studies the effect of fungicide and cultivar on yield.

Example 1

Potato yields per plot are shown in Table 1.

The three cultivars ($a = 3$) were laid out in five complete blocks ($r = 5$). The data are balanced. To simulate the effect of unbalanced data, we also consider the case where the observation for cultivar 1 in block 2 is missing. Yields are regarded as realizations of a random variable y_{ij}, which is modelled by

$$y_{ij} = \mu + \alpha_i + bl_j + e_{ij}, \qquad (1)$$

Table 1: Yields (dt ha^{-1}) of three cultivars in five blocks (balanced data)

	Block				
Cultivar	1	2	3	4	5
1	330	320[1]	335	310	315
2	315	300	320	305	320
3	300	310	320	300	305

[1] Assumed missing to simulate unbalanced data.

where μ is the general mean, α_i, the effect of i-th cultivar (fixed), bl_j, the effect of j-th block, and e_{ij}, the residual [random, $e_{ij} \sim N(0, \sigma_R^2)$].

The i-th cultivar mean is given by $\mu_i = \mu + \alpha_i$, providing sum-to-zero restrictions are imposed on the block effects bl_j. The expression '$e_{ij} \sim N(0, \sigma_R^2)$' implies that e_{ij} follows a normal distribution with zero mean and variance σ_R^2.

Example 2

The data for the rapeseed experiment are shown in Table 2. The design was a split-plot with main-plot factor fungicide ($a = 2$ levels) laid out in complete blocks and subplot factor cultivar ($b = 4$ levels) completely randomized within main-plots.

The design had four blocks ($r = 4$). There are three missing observations.

The yields are modelled as

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + bl_k + f_{ik} + e_{ijk} \qquad (2)$$

where μ is the general mean, α_i, the main effect of i-th fungicide (fixed), β_j, the main effect of j-th cultivar (fixed), $(\alpha\beta)_{ij}$, the interaction of i-th fungicide and j-th cultivar (fixed), bl_k, the effect of k-th block, f_{ik}, the main-plot error [random, $f_{ik} \sim N(0, \sigma_{RA}^2)$], e_{ijk}, the sub-plot error, i.e. residual [random, $e_{ijk} \sim N(0, \sigma_R^2)$].

The treatment mean for the i-th fungicide and the j-th cultivar is given by $\mu_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij}$, with a sum-to-zero constraint on the block effect bl_k.

Levels of the treatment factors in both experiments were purposefully selected and do not represent a distribution or population of levels. Thus, all treatment effects are regarded as fixed (Searle et al. 1992; p. 18). Modelling of the block factor is less clear-cut. Blocking is a basic principle in the design of experiments. The objective of blocking is to control heterogeneity of experimental conditions by grouping of observational units into relatively homogeneous groups. The identification of blocks does not usually involve a random element, so sampling theory cannot be invoked to justify the assumption of random block effects. When the design involves incomplete blocks, however, groups of treatments are allocated to blocks by a randomization step, and, alluding to randomization theory, this justifies the assumption of random block effects. Nevertheless, we recommend to usually take block effects as fixed, when the objective is to make inferences regarding treatment differences. This recommendation applies in particular to experiments where the number of blocks is small, regardless of whether or not the randomization scheme would justify the assumption of random effects. Our recommendation is based on a simulation study by Hu (2002), in which for a variety of designs with a small number of blocks a better control of the type I error rate was achieved under the assumption of fixed blocks, even when block effects were sampled from a normal distribution. In these cases, recovery of inter-block information (Cochran and Cox 1957), requiring the assumption of random blocks, is not worthwhile, because the block variance component cannot be estimated with sufficient accuracy. By contrast, recovery of information increases efficiency of the analysis when the number of incomplete blocks is large, as is usually the case with lattice designs (Spilke et al. 2004). It should be noted that for balanced data,

Table 2: Yield (dt ha^{-1}) of four rapeseed cultivars tested with two fungicide treatments (unbalanced data)

	Fungicide 1				Fungicide 2			
	Cultivar 1	Cultivar 2	Cultivar 3	Cultivar 4	Cultivar 1	Cultivar 2	Cultivar 3	Cultivar 4
Block 1	31.0	31.4	x	x	43.7	35.1	33.2	29.1
Block 2	31.2	28.0	28.1	33.7	40.9	33.3	34.9	30.8
Block 3	32.1	35.0	34.6	30.0	38.3	37.5	32.7	31.1
Block 4	34.8	34.7	35.2	32.8	39.2	x	32.3	31.8

statistical inference for differences of treatment means is unaffected by whether or not blocks are taken as random.

There may occasionally be an interest in confidence limits for treatment means. Random blocks usually yield wider intervals than fixed blocks. Confidence intervals based on a mixed model with random blocks have a broader inference space (McLean et al. 1991), providing blocks can be regarded as a random sample from a defined agroecological region. This latter assumption, however, is not tenable in the majority of experiments. In these cases we recommend to refrain from the computation and interpretation of confidence intervals for treatment means. We admit that our view regarding the assumption of random blocks is not the only one possible, and there are several contrasting opinions among statisticians (see Samuels et al. 1991, and discussion therein). Specifically, some statisticians regard blocks as random throughout and use best linear unbiased estimation to accommodate different 'inference spaces' (McLean et al. 1991).

Mixed model analysis of unbalanced data

The analysis of unbalanced data by mixed models differs from the analysis based on a linear model with only one independent error term in several important respects, as detailed below.

Choice of method for variance component estimation

In most applications, the variances of random effects will be unknown and need to be estimated. While for balanced data, estimates based on analysis-of-variance (ANOVA) mean squares have optimal properties (Ahrens 1967, Piepho and Spilke 1999), this is no longer true for unbalanced data. The preferable method for unbalanced data is that based on the restricted maximum likelihood (REML) approach (Searle et al. 1992), as has been verified by simulations with data structures relevant in agricultural research (Spilke and Tuchscherer 2001, Guiard et al. 2003).

Choice of method for approximating the degrees of freedom

A further complication in mixed model analysis is that the squared standard error of a contrast among fixed effects (e.g. a difference of treatment means) cannot usually be expressed as a linear function of a single variance component. For balanced data, the squared standard error is a linear function of several variance components of random effects, and the approach of Satterthwaite (1946) can be used, which entails a weighted average of the degrees of freedom (d.f.) of the mean squares associated with the random effects. This approach is not applicable, however, when the data are unbalanced and weighted least squares are used for estimation, whence the squared standard error of a contrast is a non-linear function of variance components. In this case, Wald-type *F*- and *t*-tests need to be used, which differ from standard ANOVA procedures. In addition, the REML method, which is to be preferred with unbalanced data, does not yield mean squares as required by the method of Satterthwaite (1946).

The more general approach by Giesbrecht and Burns (1985) and Fai and Cornelius (1996), which traces back to Satterthwaite (1941), is also applicable for unbalanced data. It uses the asymptotic variance–covariance matrix of variance component estimates for approximating the d.f. for Wald-type *F*- and *t*-tests. It is available by the SATTERTHWAITE option in MIXED. The approach was developed further by Kenward and Roger (1997), who incorporated a correction to reduce bias in the estimated variance–covariance matrix of fixed effects (see section 4). It is available via the KENWARDROGER option of MIXED. Hu (2002) compared via Monte Carlo simulation a large number of unbalanced designs common in agricultural research. He found the Kenward–Roger method to be preferable to other approaches, in terms of the nominal type I error rate control.

Estimation of treatment means

For balanced data, simple arithmetic treatment means have optimal properties. This does not apply to unbalanced data, where adjusted means (least square means) are preferable. Adjusted means are obtained as a linear combination of estimated fixed effects, and they guarantee an unbiased estimation of treatment means. Thus, the MIXED procedure only computes adjusted means (LSMEANS statement). It should be noted that for balanced data, adjusted means and arithmetic means are identical, but not for unbalanced data (see example 1 in section 4). Thus, adjusted least square means will always be correct, while simple means should only be used with balanced data (in which case they are identical to least square means).

Analysis of example 1

The appropriate use of the MIXED procedure is illustrated using examples 1 and 2. To avoid duplication, we do not consider all aspects with both examples. Our analysis of example 1 focuses on methods for variance component estimation, the estimation of treatment means, a comparison of means by *t*-tests, and confidence limits for means. With example 2, we exemplify the use of Wald-type *F*-tests and a macro for a compact display of multiple comparisons.

For example 1, we will compare analyses assuming random and fixed block effects. In addition, we will discuss the results of different variance component estimation methods and different methods of approximating the d.f. The analysis is based on the balanced data in Table 1. We also present results for unbalanced data, which are generated by deleting observation y_{12}.

Variance component estimates

The method of variance component estimation is specified using the METHOD option. The statement METHOD = TYPE3 invokes an ANOVA estimation method based on type III sums of squares. This method is to be preferred with balanced data always. The default method of MIXED is REML, which will yield identical results, provided all ANOVA estimates are positive (Searle et al. 1992). Otherwise, results differ, because REML estimates are constrained to be positive. The constraint can be lifted by using the NOBOUND option, whence REML and ANOVA yield identical results for balanced data. Variance component estimates are displayed in Table 3.

When the block effect is fixed, there is only one random term (residual). In this case, REML and ANOVA estimates of the residual variance component are identical. Differences occur only when data are unbalanced and blocks are random, i.e. when the model is of the mixed type. In this case, the REML method, which is the default, is preferable (Searle et al. 1992).

Estimation of treatment differences and tests of hypotheses

The approximation methods for the d.f. is specified by the DDFM option of the MODEL statement. Invocation of this option with a mixed model (random blocks) is necessary, because the default method (containment method) is not always the best choice. Simulations by Hu (2002) showed that the containment method may yield severely inflated type I error rates. For balanced data, the Satterthwaite (DDFM = SATTERTH) and Kenward–Roger (DDFM = KENWARDROGER) methods yield identical results. We recommend use of the former, because it is computationally less demanding (Appendix, programs 2 and 3). This statement is valid as long as only fixed effects are estimated. The adjustment does alter the standard errors of random effect estimates (BLUPS), even in the balanced case. When data are unbalanced due to the loss of observation y_{12} for the first cultivar, arithmetic means and adjusted means for that cultivar no longer agree (Tables 4 and 5). The same holds true for differences involving the first cultivar. The adjusted means are to be preferred in this case, because they yield unbiased estimates. The standard errors are increased compared with the balanced data. The inflation is most pronounced for cultivar 1, because this is the cultivar with the missing value.

When blocks are fixed, the DDFM option need not be invoked (Appendix, program 1), because there is just the residual variance component, and hence all methods yield identical results (Table 4). The difference among methods becomes apparent only when blocks are random. For balanced data, results for treatment differences assuming random blocks are the same as under a model with fixed blocks (Table 4). Thus, when reporting results for random block effects (Table 5), we

Table 3: Variance component estimates for balanced and unbalanced data from Table 1 assuming both fixed and random block effects (example 1)

	Balanced data		Unbalanced data		
	Blocks fixed[1]	Blocks random[2]	Blocks fixed[1]	Blocks random (ANOVA)	Blocks random (REML)
$\hat{\sigma}^2_{bl}$	–	38.75	–	40.07	39.39
$\hat{\sigma}^2_{R}$	47.92	47.92	54.02	54.02	53.38

[1] ANOVA = REML = REML NOBOUND under model with fixed block effects.
[2] ANOVA = REML NOBOUND, ANOVA = REML = REML NOBOUND when all estimates positive.

Table 4: Results of significance tests for differences of adjusted means, when blocks are fixed

	Balanced data[1]				Unbalanced data[2]			
Difference	Estimate[3]	S.E.	d.f.	$P > \|t\|$[4]	Estimate	S.E.	d.f.	$P > \|t\|$[4]
$\hat{\mu}_1 - \hat{\mu}_2$	10	4.38	8	0.05	9.4	5.06	7	0.11
$\hat{\mu}_1 - \hat{\mu}_3$	15	4.38	8	0.01	14.4	5.06	7	0.02
$\hat{\mu}_2 - \hat{\mu}_3$	5	4.38	8	0.29	5	4.65	7	0.32

[1] Estimates of effects: $\hat{\mu} = 306.7$; $\hat{\alpha}_1 = 15$; $\hat{\alpha}_2 = 5$; $\hat{\alpha}_3 = 0$.
[2] Estimates of effects: $\hat{\mu} = 306.9$; $\hat{\alpha}_1 = 14.4$; $\hat{\alpha}_2 = 5$; $\hat{\alpha}_3 = 0$.
[3] Results for arithmetic means and adjusted means always identical.
[4] Probability under H_0 that a t-distributed random variable exceeds observed $|t|$, where t = estimate/S.E.
The last treatment effect is set to zero to obtain a least squares solution of fixed effects.

Table 5: Results of significance tests for differences of adjusted means, when blocks are random, data are unbalanced, and variance components are estimated by the REML method

		DDFM = SATTERTH			DDFM = KENWARDROGER		
Difference	Estimate[1]	S.E.	d.f.	$P > \|t\|$[2]	S.E.	d.f.	$P > \|t\|$[2]
$\hat{\mu}_1 - \hat{\mu}_2$	9.83	4.98	7.43	0.087	5.04	7.43	0.090
$\hat{\mu}_1 - \hat{\mu}_3$	14.83	4.98	7.43	0.019	5.04	7.43	0.020
$\hat{\mu}_2 - \hat{\mu}_3$	5.00	4.62	7.16	0.314	4.62	7.16	0.314

Estimates of effects: $\hat{\mu} = 307$; $\hat{\alpha}_1 = 14.83$; $\hat{\alpha}_2 = 5$; $\hat{\alpha}_3 = 0$.
[1] Results for arithmetic means and adjusted means always identical.
[2] Probability under H_0 that a t-distributed random variable exceeds observed $|t|$, where t = estimate/S.E.
The last treatment effect is set to zero to obtain a least squares solution of fixed effects.

only consider the unbalanced case. In addition, we only present results based on REML estimates of variance components. The assumption of random blocks for unbalanced data effects a recovery of inter-block information (Cochran and Cox 1957; p. 460), which is why adjusted means under this model (Table 5) are not identical to those assuming fixed blocks (Table 4).

The approximated d.f. are not usually integer-valued. The correction of the standard error estimate by the Kenward–Roger method is effective for all estimates involving the first cultivar (Table 5). Generally, the correction is operative whenever the data are unbalanced, but not otherwise in case of fixed effects.

Estimation of treatment means and their confidence limits

We restrict our attention to the DDFM = KENWARDROGER option, as this was found to perform best across a broad range of designs (J. Spilke, H. P. Piepho, X. Hu, unpublished data). The choice of blocks as fixed or random factor has a pronounced effect on the width of confidence limits for a mean. Random block effects generally yield wider limits. For unbalanced data, the mean estimates and their limits exploit the inter-block information. Note that results for balanced and unbalanced data differ only for cultivar 1, for which an observation is missing in the unbalanced data (Table 6). The SAS code for the results in Table 6 may be found in the Appendix (programs 1–3). It should be reiterated that confidence limits for treatment means are meaningful only when blocks can be regarded as a random sample from a defined population of blocks, which is rarely the case in field experiments.

Analysis of example 2

Here we restrict our attention to treatment differences. The mixed model is slightly more complex than for example 1. Our presentation focuses on options for variance component estimation and approximation of the d.f. The REML estimates of variance components are $\hat{\sigma}^2_{RA} = 0.78$ and $\hat{\sigma}^2_R = 4.96$. The Wald-type *F*-tests are given in Table 7.

The significance of the interaction term (fungicide × cultivar) suggests that a comparison of marginal means for fungicides or cultivars is not useful. Instead, mean comparisons for fungicides

Table 6: Estimates of adjusted means and half widths of confidence limits around the mean (coverage probability = 95%)

	Balanced data			Unbalanced data			
		Blocks fixed	Blocks random	Blocks fixed		Blocks random	
	Estimate	Half width		Estimate	Half width	Estimate	Half width
$\hat{\mu}_1$	322	7.14	9.49	321.37	9.12	321.8	10.68
$\hat{\mu}_2$	312	7.14	9.49	312	7.77	312	9.85
$\hat{\mu}_3$	307	7.14	9.49	307	7.77	307	9.85

Table 7: Wald-type *F*-tests for example 2

Effect	Numerator d.f.	Denominator d.f.	*F*-value	P > *F*[1]
Fungicide	1	2.96	6.46	0.086
Cultivar	3	15.4	6.42	0.005
Fungicide × cultivar	3	15.5	6.09	0.006

[1] Probability under H_0 that an *F*-distributed random variable exceeds observed F.

should be carried out separately for each cultivar. Similarly, comparisons among cultivars need to be carried out separately for each fungicide (Dörfel and Bätz 1980). A few such comparisons are shown in Table 8. These were obtained using the LSMEANS statement and the option PDIFF.

In the past, a frequent problem with the use of multiple comparison procedures for unbalanced data in general and the LSMEANS statement in particular has been a lack of a compact display of mean comparisons, as is available for balanced data with the MEANS statement of the GLM and ANOVA procedures. The reason is that for unbalanced data, the standard error of a difference is not constant for all comparisons and hence there is no common critical difference. Piepho (2004) has also proposed an algorithm for obtaining a letter display for unbalanced data, which has been implemented as a SAS macro (%MULT) available from the second author upon request. The necessary SAS commands are given in the Appendix (program 4). Three additional lines are needed compared with the usual call of the MIXED procedure. The first two precede the MIXED call and save adjusted means and their differences in two temporary data files using the output delivery system (ODS). The third additional line follows the MIXED call and invokes the macro. The results for the comparison among cultivars for both fungicides are shown in Table 9. For fungicide 1, there are no differences, while for fungicide 2 the first cultivar is significantly better than the other cultivars.

Table 9: Comparison of cultivar means for two different fungicides using *t*-tests

	Fungicide	
Cultivar	1	2
1	32.28[a]	40.53[b]
2	32.28[a]	35.47[c]
3	32.49[a]	33.28[ac]
4	32.02[a]	30.70[a]

Mean values in a column followed by the same letter are not significantly different. Letters display generated using algorithm by Piepho (2004).

Conclusion

The analysis of experiments based on mixed models is readily available using the MIXED procedure. However, an appropriate analysis is not usually

Table 8: Results of LSMEANS statement (see Appendix, program 4)

	Difference	S.E.	d.f.	P > |t|[1]
Cultivar 1–cultivar 2 for fungicide 2 ($\hat{\mu}_{21} - \hat{\mu}_{22}$)	5.26	1.71	16.6	0.0071
Cultivar 1–cultivar 3 for fungicide 2 ($\hat{\mu}_{21} - \hat{\mu}_{23}$)	7.25	1.56	15.7	0.0003

[1] Probability under H_0 that a t-distributed random variable exceeds observed |t|, where t = estimate/S.E.

Table 10: METHOD and DDFM options of the MIXED procedure recommended depending on data structure (balanced or unbalanced)

Data structure	Variance component estimation	Approximation of d.f.
Balanced	METHOD = TYPE3[1]	DDFM = SATTERTHWAITE[2]
	METHOD = REML NOBOUND[1]	DDFM = KENWARDROGER[2]
Unbalanced	METHOD = REML	DDFM = KENWARDROGER

[1] Usually yield identical results for balanced data.
[2] Always yield identical results for balanced data.

obtained using the default settings. The choice of method for variance component estimation and of an approximation of the degrees of freedom is critical. In Table 10, we summarize the relevant options that should be used with balanced and with unbalanced data.

The use of the NOBOUND option in conjunction with METHOD = REML allows negative variance component estimates, which is useful when the data are balanced. In this case, the resulting variance component estimates are identical to ANOVA estimates and are optimal. For unbalanced data, however, the NOBOUND option cannot be generally recommended. Our simulation results show that with the NOBOUND option there is a high chance of obtaining no fixed-effect estimates and associated standard errors, although variance components are calculated. This is particularly true when the data set is small and the residual variance component is small compared with other variance components. The problem may occur when the variance–covariance matrix of the observation vector is not positive-definite, but current estimates are accepted nonetheless. This may happen quite frequently with unbalanced data, but is observed only rarely with balanced data.

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Appendix

Program 1

SAS statements for example 1, blocks fixed, data balanced or unbalanced, estimation of adjusted means and their differences, tests of hypotheses

```
PROC MIXED DATA = daten_1 METHOD
  = TYPE3;  /*$*/
  CLASS  cultivar block;
  MODEL  yield = cultivar block;
  LSMEANS  cultivar / PDIFF CL;
RUN;
$ Alternatives: METHOD = REML or METHOD =
REML NOBOUND.
```

Program 2

SAS statements for example 1, blocks random, balanced data, estimation of adjusted means

```
PROC MIXED DATA = daten_1 METHOD
  = TYPE3;  /*$*/
  CLASS  cultivar block;
  MODEL  yield = cultivar / DDFM = SATTERTH;
  RANDOM  block;
  LSMEANS  cultivar / CL;
RUN;
$ Alternative: METHOD = REML NOBOUND.
```

Program 3

SAS statements for example 1, blocks random, unbalanced data, estimation of adjusted means

```
PROC MIXED DATA = daten_1 METHOD = REML;
  CLASS  cultivar block;
  MODEL  yield = cultivar / DDFM = KENWARDROGER;
  RANDOM  block;
  LSMEANS  cultivar / CL;
RUN;
```

Program 4

SAS statements for example 2, fixed blocks. Invocation of macro %MULT to generate letters display for fungicide = 1.

```
ODS OUTPUT lsmeans = lsmeans;
ODS OUTPUT diffs = diffs;
PROC MIXED DATA = daten_2 METHOD = REML;
  CLASS  fungicide cultivar block;
  MODEL  yield = fungicide cultivar
  fungicide * cultivar lock / DDFM = KENWARDROGER;
  RANDOM  fungicide * block;
  LSMEANS  fungicide * cultivar / PDIFF;
RUN;
%mult (trt = cultivar, by = fungicide, level = 1);
```