Case study 4: Mixed model analysis for the estimation of components of genetic variation in lamb weaning weight

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Contents

Summary

This case study continues the analysis of differences in weaning weight between indigenous genotypes of sheep which was started in Case Study 3. In the previous case study a model containing fixed effects for lamb genotype, year of birth, sex, age at weaning and age of dam was fitted by the method of general least squares. Here we extend the model by introducing random effects for sire and dam and use the method of restricted maximum likelihood (REML) to fit the mixed model. The case study explores the multilevel structure of the data and shows how the different layers can be expressed diagrammatically in the form of a ´mixed model tree´.

The outputs produced by REML are described and compared with outputs produced by the method of general least squares. Although the presentations of results are different, analyses of variance and parameter estimates and standard errors are shown to be the same when no random terms are included in the model. Random terms for ram and ewe are then added to the statistical model. The interpretation and significance of their effects are discussed. The use of R for the analysis of these data is illustrated as well as GenStat.

Background

Helminths (parasites that reside in an animal´s intestines – see glossary of scientific terms in Case

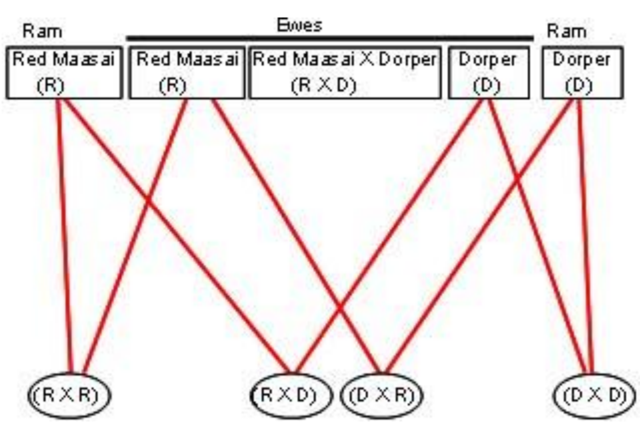
Study 3 constitute one of the most important constraints to small ruminant livestock production in the tropics resulting in widespread infection in grazing animals, associated production losses, high costs of treatment and death. Current control methods in the tropics focus on reducing contamination of pastures through anthelmintic treatment of animals and/or controlled grazing. But there are problems with increasing frequencies of drug resistance.

Source: Isaac Kosgey

An attractive, alternative and sustainable solution is the breeding for disease resistance. Indeed, anecdotal evidence suggests that among the large and diverse range of indigenous breeds of sheep and goats in the tropics there are some that appear to have the genetic ability to resist or tolerate helminthiasis. One of these is the Red Maasai breed found in East Africa and perceived to be resistant to the disease. The Red Maasai is a fat-tailed sheep associated with the Maasai tribe found in northern Tanzania and south-central Kenya.

As explained in Case Study 3, ILRI decided in 1990 to investigate the degree of resistance exhibited by this Red Maasai breed and initiated a study at Diani Estate of the Baobab Farms, 20 km south of Mombasa in the sub-humid coastal region of Kenya. To do so, a susceptible breed, the Dorper, originally from South Africa, was chosen to provide a direct comparison with the Red Maasai. The Dorper breed was developed in South Africa in the 1940s by interbreeding the Dorset Horn and Black Head Persian breeds. The Dorper is particularly well adapted to harsh, arid conditions and was imported into Kenya in the 1960s. This breed is also larger than the Red Maasai, and this makes these sheep attractive to farmers.

The design of the study is described in Case Study 3 and further details of the experimental design are given in Baker et al. (1999) and Baker et al. (2003) . Throughout six years from 1991 to 1996 Dorper (D), Red Maasai (R) and Red Maasai - Dorper crossed ewes were mated to Red Maasai and Dorper rams to produce a number of different lamb genotypes. For the purposes of this example, only the following four offspring genotypes are considered: D x D, D x R, R x D and R x R.



Case Study 3 explores the nature of associations between various factors such as age of dam and sex of offspring on weaning weight in a fixed effect least squares analysis of variance.

As well as comparing the performance of the different genotypes when exposed to helminthiasis, it is also of interest to examine genetic variation among rams and ewes within genotypes. To do this we need to use what are known as ´restricted ´ or ´residual maximum likelihood ´ (REML) procedures which are able to simultaneously estimate random and fixed effects.

Once the random estimates are known these can then be used to obtain heritability estimates which determine the proportion of the variation among offspring that has been handed down from parents.

Objectives

The objectives of the study were primarily:

- to compare the performance of the Red Maasai and Dorper breeds and their crosses in terms of their productivity under high disease risk
- to study genetic sources of variation among lambs within the two breeds and their crosses

The first objective was examined in Case Study 3 using weaning weight as one of the performance criteria.

Here we examine the second objective, namely incorporation of random effects to study variations among rams (sires) and ewes (dams) and their influences on lamb weaning weight.

Source: Isaac Kosgey

Questions to be addressed

This case study involves the use of mixed models of fixed and random effects and addresses a number of questions.

- What is a mixed model?
- How does one use REML to fit a mixed model and how can one interpret the output?
- Finally, having fitted the model how can one deduce whether there are significant random effects of ram and ewe on offspring weaning weight?

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How does one use REML to fit a mixed model and how can one interpret the output?

Finally, having fitted the model how can one deduce whether there are significant random effects of ram and ewe on offspring weaning weight?

- In addressing these questions the case study first considers the statistical model with just the fixed effects developed in Case Study 3 and compares the outputs obtained by the conventional method of general least squares with that using REML.
- The case study then dwells at some length on the meaning of a mixed model, how it can incorporate units of observation at different layers and how the data structure framework can be diagrammatically sketched in the form of a ´mixed model tree´.
- Finally, having fitted the mixed model, the case study describes how to interpret the findings.

Source material

The data set used in this example is stored in the Excel file CS4Data. This is the same data as used in Case Study 3 with minor changes in the variable field names. The fields are described in the associated word file CS4Doc. These fields include both data collected during the study and others derived during the earlier statistical analysis.

A number of variables (both original and derived) have already been defined as factors. The records without entries for weaning weight, either because the lamb died prior to weaning or because recording was missed, will be omitted from the analysis

Note that the first 4 rows of CS4Data contain the documentation for the data, so these need to be ignored when opening the file in GenStat.

Exploration & description

Contents

Fixed and random effects
Observational units
ANOVA or REML?

Before incorporating ram and ewe random effects into the statistical model it is worth discussing first the meaning of mixed models.

Mixed model methodology takes its name from the understanding that the elements of the model underlying a statistical analysis can be a mixture of what are called fixed and random effects. The approach has become important in the analysis of data that have a hierarchical structure, since the different layers in the structure can be modelled using random effects.

A fundamental step in using mixed models for hierarchical data is to recognise the structure, namely the different layers in the data. In order to help with this we shall use what we describe as a ´mixed model tree´ to develop the different layers pictorially. This is also illustrated in the statistical guide by Allan and Rowlands (2001) which uses the data from this case study for one of its examples.

This guide is no.19 of the Good Practice Guides. It also includes examples from Case Study 6 and from the paper by Methu et al (2001).

Exploration & description/Fixed and random effects

A random effect is a component of the data that has a degree of randomness associated with it, whereas a fixed effect has no random connotation.

An example of a fixed effect in this case study would be the sex of a lamb. It is fixed because it can only have one of two values: male and female.

On the other hand, the influence of the ram on the growth of its offspring is usually considered to be a random effect. In making this assumption the researcher assumes that the sample of rams used in the study is a random selection of rams from the particular genotype at large.

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.

Thus, in this study, the rams are regarded as a random representation of rams from Red Maasai and Dorper breeds. If such an effect is defined as random then any interaction involving the effect and any other effect, fixed or random, will also be random – and this has implications for the inferences made from the data. For instance if year is also declared random in this model, and the breed * year interaction is included in the analysis, then any inferences made about breed will be for the population of years which our sample is deemed to represent. If the breed * year interaction is not included, or if year is regarded as a fixed effect, then inferences will apply to the performance of the breeds only across the six years in question.

Exploration & description/Fixed and random effects

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An example of a fixed effect in this case study would be the sex of a lamb. It is fixed because it can only have one of two values: male and female.

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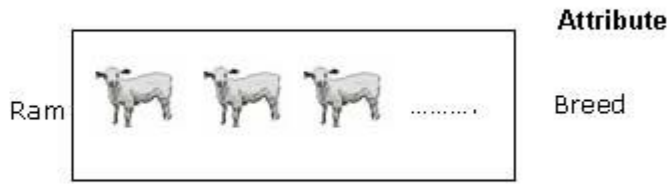
The choice of whether an effect such as breed is fixed or random is not always obvious. In this example there are only two breeds of ram and so it would not be sensible to infer that these two breeds are a random sample from a much larger population of ram breeds. This is not only because they were specifically chosen for this study, but also because a sample of two would not be considered large enough to generalise to "all breeds". Here the possibility of year being random might also been considered. Six levels, as here, are probably about the minimum number that could be considered as adequate for estimating random components. Thus, for a study carried out over only three or four years, the sample would be hardly large or random enough to be representative of a wider population of years.

In mixed model analysis we have different types of units occurring at different layers – namely in this example: lambs, ewes, rams. The investigational or observational units defined within layers are assumed to be chosen independently of one another; usually they are chosen at

´random´. They will therefore be random effects in our mixed model.

Correctly identifying the layers of observational units and the different attributes assigned to units is crucial to a successful understanding of how hierarchical data should be analysed. This is what we aim to do with our mixed model tree.

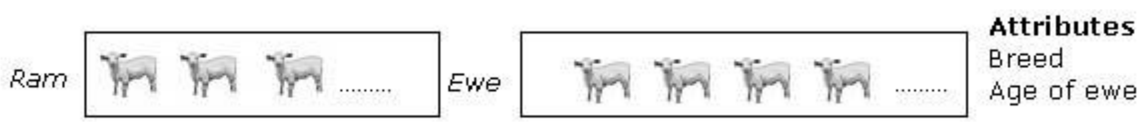
We have two breeds. From within each of the two breeds a number of rams is selected. These are the observational units (ram shown as a random effect) against which the two breeds of rams should be compared.



Exploration & description/Observational units

In mixed model analysis we have different types of units occurring at different layers – namely in this example: lambs, ewes, rams. The investigational or observational units defined within layers are assumed to be chosen independently of one another; usually they are chosen at ´random´. They will therefore be random effects in our mixed model.

We can do exactly the same for ewes. As the selection process is being carried out at the same time as the rams, the mixed model tree is formed in parallel.

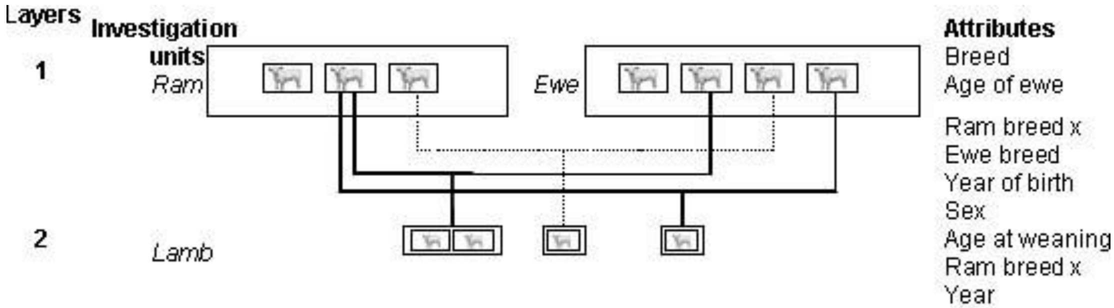


A ewe's age is an attribute that we feel may influence weaning weight.

In mixed model analysis we have different types of units occurring at different layers – namely in this example: lambs, ewes, rams. The investigational or observational units defined within layers are assumed to be chosen independently of one another; usually they are chosen at ´random´. They will therefore be random effects in our mixed model.

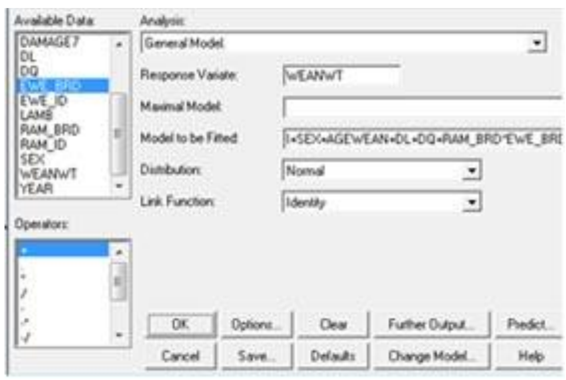
Rams and ewes are mated both within and across breeds to produce their offspring. These offspring are the investigational units at the next layer down shown together with a list of fixed effects or attributes that might be considered for each lamb.

Breed differences, however, are assessed relative to the average variation among rams and ewes within breed at the top layer.



Exploration & description/ANOVA or REML?

Before using the REML method to estimate genetic variance components we shall rerun the analysis used in **Case Study 3** to compare weaning weights of lambs. First we must disregard the lambs for which the response variable weaning weight was not recorded. This can be achieved by using the GenStat **Spread → Restrict/Filter By Value...** command and excluding missing values (*) for weaning weight



***** Regression Analysis *****

Response variate: WEANWT

Fitted terms: Constant + YEAR + SEX + AGEWEAN + DL + DQ + RAM_BRD + EWE_BRD + RAM_BRD.EWE_BRD

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.
+ YEAR	5	1208.149	241.630	48.92
+ SEX	1	55.983	55.983	11.34
+ AGEWEAN	1	344.206	344.206	69.69
+ DL	1	151.513	151.513	30.68
+ DQ	1	275.795	275.795	55.84
+ RAM_BRD	1	44.881	44.881	9.09
+ EWE_BRD	1	30.223	30.223	6.12
+RAM_BRD. EWE_BRD	1	0.754	0.754	0.15
Residual	687	3392.947	4.939	
Total	699	5504.450	7.875	

This time we shall alter the way that breed genotypes are defined in the least squares analysis. Instead of referring to the breeds by their genotype, D X D, D X R, R X D and R X R we shall consider separate effects for ram breed, ewe breed and their interaction, and re-parameterise the model accordingly. We can run this model both by least squares analysis of variance and by REML.

Let us first consider the least squares approach. Using **Stats →**

Regression Analysis → Generalized Linear Models and completing the dialog box as shown and clicking the **Options...** button and then ticking 'Accumulated', we obtain the analysis of variance indicating that the breed of ram x breed of ewe is insignificant (variance ratio = 0.15).

The output on the right shows the results for the least squares analysis of variance and parameter estimates but without interaction.

We shall next run the model through the REML procedure (**Stats → Mixed Models (REML)→ Linear Mixed Models**) and compare with that obtained by least squares analysis of variance.

A description of how the REML analysis can be conducted in R is illustrated in Mbunzi and Nagda (2009).

*** Regression ***
Response variate: WEANWT
Fitted terms: Constant + YEAR + SEX + AGEWEAN + DL + DQ + RAM_BRD + EWE_BRD

Estimates of parameters

	Estimate	s.e.	t(688)	tpr.
Constant	12.95	1.07	0.26	0.797
YEAR 92	-1.566	0.293	-5.35	<.001
YEAR 93	-1.096	0.275	-3.98	<.001
YEAR 94	-2.833	0.358	-7.92	<.001
YEAR 95	-3.228	0.344	-9.39	<.001
YEAR 96	-2.351	0.390	-6.03	<.001
SEX M	0.478	0.169	2.82	0.005
AGEWEAN	0.07022	0.00886	7.93	<.001
DL	2.726	0.315	8.65	<.001
DQ	-0.2689	0.0340	-7.91	<.001
RAM_BRD R	-0.443	0.173	-2.56	0.011
EWE_BRD R	-0.586	0.237	-2.48	0.014

***Accumulated analysis of variance ***

Change	d.f	s.s.	m.s.	v.r.
+ YEAR	5	1208.149	241.630	48.99
+ SEX	1	55.983	55.983	11.35
+ AGEWEAN	1	344.206	344.206	69.78
+ DL	1	151.513	151.513	30.72
+ DQ	1	275.795	275.795	55.19
+ RAM_BRD	1	44.881	44.881	9.10
+ EWE_BRD	1	30.223	30.223	6.13
Residual	688	3393.701	4.933	
Total	699	5504.450	7.875	

For REML we need to click the Options button in the dialogue box to ensure that the items 'Model', 'Variance components', 'Estimated effects', 'Stratum variances', 'Deviance' and 'Wald Tests' are included for display; we also need to ensure that the 'Fisher scoring method' is clicked for the 'Optimisation method' as this is necessary for calculating stratum variances.

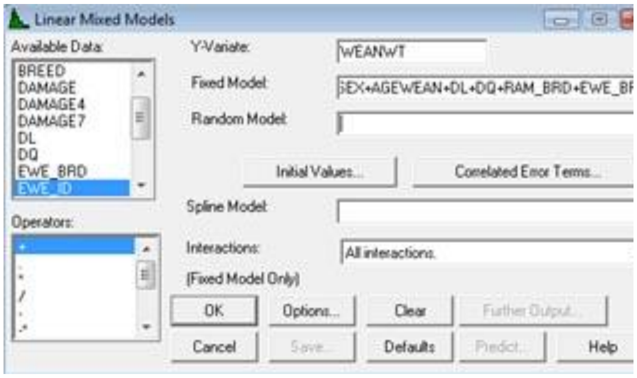
***** REML Variance Components Analysis *****

Response Variate : WEANWT

*** Approximate stratum variances ***
Effective d.f.
units 4.933 688.00

*** Wald tests for fixed effects ***

Fixed Wald statistic d.f. Wald/d.f. Chi-sq prob



term
* Sequentially adding terms to fixed model

YEAR	244.93	5	48.99	<0.001
SEX	11.35	1	11.35	<0.001
AGEWEAN	69.78	1	69.78	<0.001
DL	30.72	1	30.72	<0.001
DQ	55.91	1	55.91	<0.001
RAM_BRD	9.10	1	9.10	0.003
EWE_BRD	6.13	1	6.13	0.013

By comparing the outputs from the previous two slides it can be seen that both the least squares analysis and the REML analysis without a random term obtain the same solutions (compare 'v.r.' and 'Wald/d.f.'). Just the format of the output is different. (Later we list the REML parameter estimates and associated standard errors; as will be seen these are the same as those from the least squares analysis.)

GenStat calculates values known as Wald statistics instead of F-values for a mixed model. The Wald test investigates the same hypotheses as the F test in the least squares analysis of variance – i.e. null hypothesis of no effect - but unlike the F-statistic, which follows an F-distribution, the Wald statistic follow a Chi-square distribution, but only approximately.

Significance levels tend to be a little lower for the Wald test than for the F test when random terms are included, and this will, by and large, always be the case unless the sample size, as here, is comparatively large.

***** REML Variance Components Analysis *****

Response Variate : WEANWT

*** Approximate stratum variances ***
Effective d.f.
units 4.933 688.00

*** Wald tests for fixed effects ***

Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
* Sequentially adding terms to fixed model				
YEAR	244.93	5	48.99	<0.001
SEX	11.35	1	11.35	<0.001
AGEWEAN	69.78	1	69.78	<0.001
DL	30.72	1	30.72	<0.001
DQ	55.91	1	55.91	<0.001
RAM_BRD	9.10	1	9.10	0.003
EWE_BRD	6.13	1	6.13	0.013

To derive the corresponding F-values from the values of the Wald statistics one just needs to divide the Wald statistic by

***** REML Variance Components Analysis *****

the corresponding degrees of freedom. These values are shown here under the column headed Wald/d.f.

The v.r. or F value for YEAR in the least squares analysis of variance shown further down is 48.99; this is the same as the corresponding Wald/d.f. value shown alongside.

Note also that the estimated residual variance is the same in both outputs, i.e. *units* stratum variance = 4.933 with 688 degrees of freedom, which is the same as the residual m.s. value in the least squares analysis of variance.

```
Response Variate : WEANWT

*** Approximate stratum variances ***
Effective d.f.
*units* 4.933 688.00
*** Wald tests for fixed effects ***
Fixed          Wald statistic  d.f.  Wald/d.f. Chi-sq prob
term
* Sequentially adding terms to fixed model

YEAR                 244.93     5      48.99      <0.001
SEX                   11.35     1      11.35      <0.001
AGEWEAN               69.78     1      69.78      <0.001
DL                    30.72     1      30.72      <0.001
DQ                    55.91     1      55.91      <0.001
RAM_BRD                9.10     1       9.10       0.003
EWE_BRD                6.13     1       6.13       0.013
```

Least Squares analysis

```
Accumulated analysis of variance
Change        d.f        s.s.       m.s.         v.r.
+ YEAR          5    1208.149    241.630        48.99
+ SEX           1      55.983     55.983        11.35
+ AGEWEAN       1     344.206    344.206        69.78
+ DL            1     151.513    151.513        30.72
+ DQ            1     275.795    275.795        55.19
+ RAM_BRD       1      44.881     44.881         9.10
+ EWE_BRD       1      30.223     30.223         6.13
Residual      688    3393.701      4.933

Total         699    5504.450      7.875
```

The table for the Wald tests shows that the main effects of ram breed and ewe breed, when adjusted for ram breed, are significant with Wald statistics of 9.10 and 6.13. when compared with Chi-square values with 1 degree of freedom, P=0.003 and 0.013, respectively.

Although not shown, virtually the same P-values are obtained when applying F-tests to the mean squares in the least squares analysis of variance.

```
***** REML Variance Components Analysis
*****
Response Variate : WEANWT

*** Approximate stratum variances ***
Effective d.f.
*units* 4.933 688.00
*** Wald tests for fixed effects ***
Fixed          Wald statistic  d.f.  Wald/d.f. Chi-sq prob
term
* Sequentially adding terms to fixed model

YEAR                 244.93     5      48.99      <0.001
SEX                   11.35     1      11.35      <0.001
AGEWEAN               69.78     1      69.78      <0.001
DL                    30.72     1      30.72      <0.001
DQ                    55.91     1      55.91      <0.001
RAM_BRD                9.10     1       9.10       0.003
EWE_BRD                6.13     1       6.13       0.013
```

Least Squares analysis

```
Accumulated analysis of variance
Change        d.f        s.s.       m.s.         v.r.
+ YEAR          5    1208.149    241.630        48.99
+ SEX           1      55.983     55.983        11.35
```

+ AGEWEAN	1	344.206	344.206	69.78
+ DL	1	151.513	151.513	30.72
+ DQ	1	275.795	275.795	55.19
+ RAM_BRD	1	44.881	44.881	9.10
+ EWE_BRD	1	30.223	30.223	6.13
Residual	688	3393.701	4.933	
Total	699	5504.450	7.875	

The Wald statistics also demonstrate the highly significant fixed effects of year of birth, age of ewe (DL and DQ), age at weaning and sex, as shown earlier by least squares analysis of variance.

The table of effects shown alongside demonstrates, for example, that the lambs born in the later years had lower weaning weights compared with those born in the earlier years and, further, that male lambs had an average weaning weight slightly higher by 0.4779 (± 0.1695) kg than females.

```
*** Table of effects for YEAR ***
```

YEAR	91.00	92.00	93.00	94.00	95.00	96.00
	0.000	-1.566	-1.096	-2.833	-3.228	-2.351

```
Standard error of differences:          Average   0.3373
                                        Maximum   0.3898
                                        Minimum   0.2753
Average variance of differences:                  0.1151

*** Table of effects for SEX ***
SEX            F              M
               0.0000         0.4779
Standard error of differences: 0.1695

** Table of effects for AGEWEAN ***
0.07022 Standard error: 0.008856

*** Table of effects for DL ***
2.726 Standard error: 0.3150

*** Table of effects for DQ ***
-0.2689 Standard error: 0.03401

** Table of effects for RAM_BRD ***
RAM_BRD D R
0.0000 -0.4429

Standard error of differences: 0.1728

** Table of effects for EWE_BRD ***
EWE_BRD D R
0.0000 -0.5855

Standard error of differences: 0.2366
```

The main points to note when interpreting REML outputs are (a) the validity of the Wald test depends on the size of the sample, and (b) that the Wald test is more liberal than the F test, with the significance levels of the two becoming closer as the sample size increases.

Some statistical packages apply an F test to the Wald/d.f. value rather than a Chi-square test to the Wald statistic. Nevertheless, the above comments still


```
*** Table of effects for YEAR ***
```

YEAR	91.00	92.00	93.00	94.00	95.00	96.00
	0.000	-1.566	-1.096	-2.833	-3.228	-2.351

```
Standard error of differences:          Average   0.3373
                                        Maximum   0.3898
                                        Minimum   0.2753
Average variance of differences:                  0.1151

*** Table of effects for SEX ***
SEX            F              M
               0.0000         0.4779
Standard error of differences: 0.1695

** Table of effects for AGEWEAN ***
0.07022 Standard error: 0.008856

*** Table of effects for DL ***
2.726 Standard error: 0.3150
```

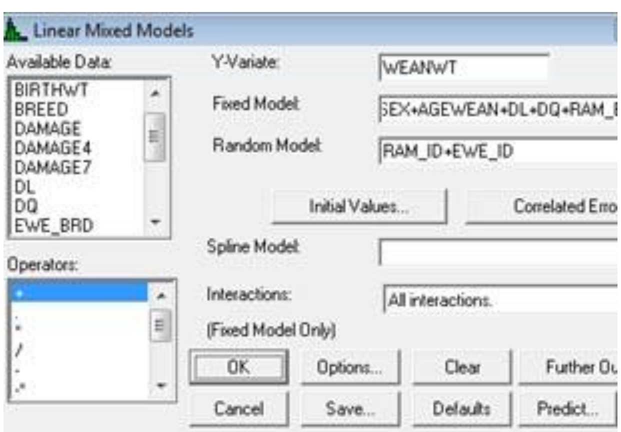
apply and the user needs to take care in calculating significance values for F-tests in a mixed model analysis.

```
*** Table of effects for DQ ***
-0.2689 Standard error: 0.03401

** Table of effects for RAM_BRD ***
RAM_BRD D R
0.0000 -0.4429

Standard error of differences: 0.1728

** Table of effects for EWE_BRD ***
EWE_BRD D R
0.0000 -0.5855

Standard error of differences: 0.2366
```

Statistical modelling

The dialog box below shows how to produce the mixed model analysis for lamb weaning weight with ram and ewe defined as random effects.

Note that since each ram and ewe is identified with its own unique code (see CS4Data), GenStat recognises that the random components are to be calculated within breed. Had ram and ewe been coded within breed (e.g. each breed with cumulative integers from 1 onwards) the random terms would have had to be specified as RAM_BRD.RAM_ID, EWE_BRD.EWE_ID, interpreted as ram within ram breed and ewe within ewe breed, respectively.




```
**Estimated Variance Components **
Random term     Component     S.e.
RAM_ID          0.067         0.089
EWE_ID          1.457         0.283

*** Residual variance model ***
Parameter          Estimate      S.e.
Sigma2                3.427     0.266

**Approximate stratum variances ***
                                            Effective d.f.
RAM_ID                   4.733                    57.66
EWE_ID                   6.490                   297.74
*units*                  3.427                   332.60

* Matrix of coefficients of components for each stratum
RAM_ID          10.31       0.42       1.00
EWE_ID           0.00       2.10       1.00
*units*          0.00       0.00       1.00

*** Deviance: -2*Log-Likelihood ***
Deviance     d.f.
1817.10      685

*** Wald tests for fixed effects ***
Fixed term        Wald statistic        d.f.          Wald/d.f.
* Sequentially adding terms to fixed model
              YEAR          230.32          5              46.06
SEX                           9.66          1               9.66
AGEWEAN                      63.84          1              63.84
DL                           30.44          1              30.44
DQ                           78.41          1              78.41
RAM_BRD                       6.64          1               6.64
EWE_BRD                       2.91          1               2.91
```

This output differs from the one given earlier in that the error variance is now shared between the random terms specified in the model. This ensures that different fixed estimates are evaluated using standard errors that have been calculated using the residual variations associated with the appropriate layer(s).

Note that compared with the earlier analysis the Wald statistics for ram breed and ewe breed have been reduced from 9.10 and 6.13, respectively, to the values 6.64 and 2.91; indeed the effect of ewe breed is no longer significant.

```
**Estimated Variance Components **
Random term      Component      S.e.
RAM_ID           0.067          0.089
EWE_ID           1.457          0.283

*** Residual variance model ***
Parameter        Estimate       S.e.
Sigma2              3.427      0.266

**Approximate stratum variances ***
                                      Effective d.f.
RAM_ID                4.733                57.66
EWE_ID                6.490               297.74
*units*               3.427               332.60

* Matrix of coefficients of components for each stratum
RAM_ID          10.31      0.42      1.00
EWE_ID           0.00      2.10      1.00
*units*          0.00      0.00      1.00

*** Deviance: -2*Log-Likelihood ***
Deviance     d.f.
1817.10      685

*** Wald tests for fixed effects ***
Fixed term        Wald statistic      d.f.        Wald/d.f.     Chi-sq prob
* Sequentially adding terms to fixed model
               YEAR        230.32        5            46.06          <0.001
SEX                          9.66        1             9.66           0.002
AGEWEAN                     63.84        1            63.84          <0.001
DL                          30.44        1            30.44          <0.001
DQ                          78.41        1            78.41          <0.001
RAM_BRD                      6.64        1             6.64           0.010
EWE_BRD                      2.91        1             2.91           0.088
```

Another notable difference between this output and the one given earlier is the addition of estimated values for variance components attributable to ram and ewe. A variance component provides a measure of the variation directly associated with the random effect itself.

Variance components have an important use because they provide the basis for calculating genetic parameters such as heritability

In this analysis the ewe variance component is higher than the ram component indicating the ewe has a significant maternal influence on its lamb's growth to weaning. On the


```
**Estimated Variance Components **
Random term      Component      S.e.
RAM_ID           0.067          0.089
EWE_ID           1.457          0.283

*** Residual variance model ***
Parameter        Estimate       S.e.
Sigma2              3.427      0.266

**Approximate stratum variances ***
                                      Effective d.f.
RAM_ID                4.733                57.66
EWE_ID                6.490               297.74
*units*               3.427               332.60

* Matrix of coefficients of components for each stratum
RAM_ID          10.31      0.42      1.00
EWE_ID           0.00      2.10      1.00
*units*          0.00      0.00      1.00

*** Deviance: -2*Log-Likelihood ***
Deviance     d.f.
1817.10      685

*** Wald tests for fixed effects ***
Fixed term        Wald statistic      d.f.        Wald/d.f.     Chi-sq prob
```

other hand the ram estimates is less than its standard error.

```
* Sequentially adding terms to fixed model
               YEAR        230.32        5            46.06          <0.001
SEX                          9.66        1             9.66           0.002
AGEWEAN                     63.84        1            63.84          <0.001
DL                          30.44        1            30.44          <0.001
DQ                          78.41        1            78.41          <0.001
RAM_BRD                      6.64        1             6.64           0.010
EWE_BRD                      2.91        1             2.91           0.088
```

The matrix of coefficients multiplied by the corresponding estimated variance component values gives the table of approximate stratum variances (equivalent to the residual mean squares in a least squares analysis of variance).

The matrix of coefficients shows that each ram sired on average just over 10 lambs. The RAM_ID stratum variance also includes a proportion (0.42) of the ewe variance component reflecting the fact that rams were mated to more than one ewe.

The 2.10 matrix coefficient for EWE_ID shows that ewes had an average of just over two offspring during the study. Since a ram was never mated to the same ewe twice, the ewe stratum variance is independent of ram (indicated by the 0.00 value for ram).

```
**Estimated Variance Components **
Random term     Component     S.e.
RAM_ID          0.067         0.089
EWE_ID          1.457         0.283

*** Residual variance model ***
Parameter    Estimate     S.e.
Sigma2       3.427        0.266

**Approximate stratum variances ***
                              Effective d.f.
RAM_ID     4.733              57.66
EWE_ID     6.490              297.74
*units*    3.427              332.60

* Matrix of coefficients of components for each stratum
RAM_ID     10.31      0.42      1.00
EWE_ID     0.00       2.10      1.00
*units*    0.00       0.00      1.00

*** Deviance: -2*Log-Likelihood ***
Deviance     d.f.
1817.10      685

*** Wald tests for fixed effects ***
Fixed term        Wald statisticd.f.      Wald/d.f.        Chi-sq prob
* Sequentially adding terms to fixed model
YEAR              230.32      5           46.06            <0.001
SEX               9.66        1           9.66             0.002
AGEWEAN           63.84       1           63.84            <0.001
DL                30.44       1           30.44            <0.001
DQ                78.41       1           78.41            <0.001
RAM_BRD           6.64        1           6.64             0.010
EWE_BRD           2.91        1           2.91             0.088
```

The table alongside, together with the equations, shows how the estimated variance components, indicated by s^2_r s^2_d and s^2_e respectively, are derived from the matrix coefficient and stratum variance values – see GenStat output on previous page.

	s_r^2 (ram)	s_d^2 (ewe)	s_e^2 (lamb)	Stratum variance
	0.067	1.457	3.427	
Coefficients				
Ram	10.31	0.42	1	4.733
Ewe	0	2.10	1	6.490
Residual	0	0	1	3.427

Ram mean square = 10.31 x s_r^2 + 0.42 x s_d^2 + 1 x s_e^2 = 4.733

Ewe mean square = 2.10 x s_d^2 +1 x s_e^2 = 6.490

Residual (lamb) mean square = 1 x s_e^2 = 3.427

With the random terms specified in the model the estimate of the residual among lamb variance is reduced from 4.939 to 3.427 kg^2, and also the effective degrees of freedom from 688 to 332.60.

This is due to taking into account the variations among rams and ewes within breeds, whereas the earlier output assumes all variation to be at the lamb level.

Therefore, the REML analysis with the random model describes more accurately the different layers of variation associated with the hierarchical data and provides a more appropriate and correct analysis.

```
**Estimated Variance Components **
Random term     Component      S.e.
RAM_ID          0.067          0.089
EWE_ID          1.457          0.283

*** Residual variance model ***
Parameter          Estimate        S.e.
Sigma2                3.427      0.266

**Approximate stratum variances ***
                                          Effective d.f.
RAM_ID                4.733                     57.66
EWE_ID                6.490                    297.74
*units*               3.427                    332.60

* Matrix of coefficients of components for each stratum
RAM_ID          10.31       0.42       1.00
EWE_ID           0.00       2.10       1.00
*units*          0.00       0.00       1.00

*** Deviance: -2*Log-Likelihood ***
Deviance     d.f.
1817.10      685

*** Wald tests for fixed effects ***
Fixed term        Wald statistic      d.f.       Wald/d.f.       Chi-sq prob
* Sequentially adding terms to fixed model
                YEAR        230.32        5          46.06          <0.001
SEX                           9.66        1           9.66           0.002
AGEWEAN                      63.84        1          63.84          <0.001
DL                           30.44        1          30.44          <0.001
DQ                           78.41        1          78.41          <0.001
RAM_BRD                       6.64        1           6.64           0.010
EWE_BRD                       2.91        1           2.91           0.088
```

Comparison of the RAM_ID and EWE_ID variance components with their standard errors indicates that the variance component for ewes (1.457) is highly significant (component > 5 times its standard error) but that for ram (0.067) is not (component less than its standard error).

This, therefore, shows how,

```
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Random term     Component      S.e.
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EWE_ID          1.457          0.283

*** Residual variance model ***
Parameter          Estimate        S.e.
Sigma2                3.427      0.266

**Approximate stratum variances ***
                                          Effective d.f.
RAM_ID                4.733                     57.66
EWE_ID                6.490                    297.74
*units*               3.427                    332.60
```

especially with the ewe component included, the mixed model utilises more of the information contained within the data than the model without the ram and ewe components.

```
* Matrix of coefficients of components for each stratum
RAM_ID        10.31     0.42     1.00
EWE_ID         0.00     2.10     1.00
*units*        0.00     0.00     1.00

*** Deviance: -2*Log-Likelihood ***
Deviance     d.f.
1817.10      685

*** Wald tests for fixed effects ***
Fixed term        Wald statistic     d.f.     Wald/d.f.     Chi-sq prob
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            YEAR        230.32        5         46.06         <0.001
SEX                       9.66        1          9.66          0.002
AGEWEAN                  63.84        1         63.84         <0.001
DL                       30.44        1         30.44         <0.001
DQ                       78.41        1         78.41         <0.001
RAM_BRD                   6.64        1          6.64          0.010
EWE_BRD                   2.91        1          2.91          0.088
```

Another way to compare models is to use what is known as ´deviance´. In simple terms this gives an overall measure of how well the model fits the data. It is calculated as − 2 times what is known as the log likelihood.

Whilst the method of least squares is the method usually adopted when fitting models involving fixed effects only, the method of maximum likelihood is the method used by REML. This method calculates an expression known as the likelihood, which measures how well the model fits the data. The better the fit to the data the smaller is the value of the −2 log likelihood. By comparing the deviance values derived from separate models one can determine which model provides a better fit to the data.

In this example the deviance statistic can be used to determine whether the model that specifies the separate variance components fits the data better than the fixed model that contains only the residual variance component.

```
**Estimated Variance Components **
Random term    Component     S.e.
RAM_ID         0.067         0.089
EWE_ID         1.457         0.283

*** Residual variance model ***
Parameter         Estimate      S.e.
Sigma2               3.427     0.266
```

The deviance in this output, namely 1817.10, is lower than the model without random effects (namely 1855.50 seen by rerunning this model). The difference between the two values: 1855.80 - 1817.10 = 38.70 with 688 − 686 = 2 degrees of freedom. This approximates to a Chi-square distribution (Chi-square (df = 2) = 38.70).

This is significant (P<0.001) and shows that the mixed model provides a better fit than the fixed effects model without the ram and ewe variance components.

```
**Approximate stratum variances ***
                                          Effective d.f.
RAM_ID                4.733                  57.66
EWE_ID                6.490                 297.74
*units*               3.427                 332.60

* Matrix of coefficients of components for each stratum
RAM_ID          10.31      0.42      1.00
EWE_ID           0.00      2.10      1.00
*units*          0.00      0.00      1.00

*** Deviance: -2*Log-Likelihood ***
Deviance     d.f.
1817.10      685

*** Wald tests for fixed effects ***
Fixed term       Wald statistic      d.f.      Wald/d.f.      Chi-sq prob
* Sequentially adding terms to fixed model
            YEAR         230.32         5         46.06           <0.001
SEX                        9.66         1          9.66            0.002
AGEWEAN                   63.84         1         63.84           <0.001
DL                        30.44         1         30.44           <0.001
DQ                        78.41         1         78.41           <0.001
RAM_BRD                    6.64         1          6.64            0.010
EWE_BRD                    2.91         1          2.91            0.088
```

Findings, implications and lessons learned

- This example has shown how mixed models can deal effectively with different layers in the data; this form of analysis gives more valid significance tests and provides appropriate and correct standard errors − something that conventional least squares analysis of variance methods cannot do except in one or two very specific circumstances.
- REML has the ability, particularly with unbalanced data structures, to combine information from the different data layers. This has the advantage of improving the precision of fixed effect comparisons.
- Before applying mixed models it may sometimes be helpful to evaluate some of the important fixed effects first (as was done in Case Study 3), and then to add the random terms later, as has been done here.
- This case study has also shown how to recognise the structures of the different layers in a data set, and has explained the understanding of variance components associated with random effects.

Reporting

When the ´Option´ **Predicted means** is specified within the Genstat dialogue box a set of least squares means can be obtained as shown below.

Table of predicted means for YEAR

YEAR	91.00	92.00	93.00	94.00	95.00	96.00
	12.64	11.07	11.56	9.64	9.35	10.19

Standard error of differences:	Average	0.3226
	Maximum	0.3947
	Minimum	0.2590
Average variance of differences:		0.1056

***Table of predicted means for SEX**

SEX	F	M
	10.54	10.94
Standard error of differences:	0.1623	

*** Table of predicted means for RAM_BRD ***

RAM_BRD	D	R
	10.95	10.54
Standard error of differences:	0.1756	

*** Table of predicted means for EWE_BRD ***

EWE_BRD	D	R
	10.97	10.51
Standard error of differences:	0.2665	

By comparing with the earlier model without random effects for ram and ewe it can be seen that the standard errors for year and sex have changed little. These are fixed effects associated with the lowest layer in the fitted model.

With the reduction in residual variance one might have expected standard errors to be reduced too. This will often be so.

However, there is considerable imbalance in the way that ewes are distributed among genotype groups and years, as illustrated in Case Study 3, and this is probably reflected in the calculation of the standard errors.

Variable	No.	Mean[a]	Mean[b]
Ram breed			
Dorper	433	10.95	11.05
Red Maasai	439	10.54	10.61
S.E.D.		0.18	0.17
Ewe breed			
Dorper	544	10.97	11.12
Red Maasai	338	10.51	10.54
S.E.D		0.27	0.24
Year			
1991	144	12.64	12.67
1992	109	11.07	11.11
1993	168	11.56	11.58
1994	79	9.64	9.84
1995	107	9.35	9.45
1996	93	10.19	10.32
S.E.D.		0.32	0.33
Sex			
Female	323	10.54	10.59
Male	377	10.94	11.07
S.E.D.		0.16	0.17

a - without random effects
b - with random effects

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1995	107	9.35	9.45
1996	93	10.19	10.32
S.E.D.		0.32	0.33
Sex			
Female	323	10.54	10.59
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a - without random effects
b - with random effects

With the reduction in residual variance one might have expected standard errors to be reduced too. This will often be so.

However, there is considerable imbalance in the way that ewes are distributed among genotype groups and years, as illustrated in Case Study 3, and this is probably reflected in the calculation of the standard errors.

There are, however, slight differences in the revised least squares means themselves for year and sex.

The standard error for ewe breed is higher than that for ram breed, reflecting the larger variance component for ewes.

Variable	No.	Mean[a]	Mean[b]
Ram breed			
Dorper	433	10.95	11.05
Red Maasai	439	10.54	10.61
S.E.D.		0.18	0.17
Ewe breed			
Dorper	544	10.97	11.12
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1994	79	9.64	9.84
1995	107	9.35	9.45
1996	93	10.19	10.32
S.E.D.		0.32	0.33
Sex			
Female	323	10.54	10.59
Male	377	10.94	11.07
S.E.D.		0.16	0.17

a - without random effects
b - with random effects

Study questions

1. Define what is meant by a mixed model. Think of an example of a set of data that might be suitable for analysis as a mixed model. Illustrate the data structure and explain which affects you would define as random terms in the model.
2. Write down the statistical model for a balanced split-plot design and decide whether it falls into the category of a mixed model or not. Similarly consider a hierarchical or nested model without any fixed effects. Does this come under the definition of a mixed model?

   Before REML procedures were available, statistical analysis of hierarchical data sets was often undertaken in two stages: first, least squares analysis of variance to estimate fixed effects, then a nested analysis of variance, with the data corrected for fixed effects to estimate random effects at each layer in the hierarchy. What do you think are the advantages of REML over this approach? Describe an example when correcting data for fixed effects may still be useful (see, for example Case Study 2). Describe how you would 'correct' the data.
3. Run GenStat with fixed effects for BREED (lamb breed), SEX, AGEWEAN, DL and DQ: a) with YEAR also as a fixed effect, b) YEAR as a fixed effect and RAM_ID and EWE_ID added as random effects and c) YEAR, RAM_ID and EWE_ID added as random effects. Compare the parameters obtained for BREED in the three outputs. Comment on how and why they vary and suggest which model you would use to report the results. Discuss the premise that year can be assumed to be a random effect.

4. Choose model b) in Question 4. Write a short report including a description of the statistical analysis (with the statistical model expressed algebraically) and a summary of the results.
5. Explain what is meant by fixed and random effects. Give an example of a split-plot design. Describe the random effects that feature in its analysis. Why can these random effects be estimated by a conventional least squares analysis of variance so that REML is not necessary in this case?
6. Yields from a number of plots are missing in a split-plot design. Describe two alternative ways of analysing the data and say when you think one method is preferable to the other.
7. What do you understand by 'heritability'? The formula for calculating an estimate of heritability from a number of offspring nested within sires is 4ss2 / (ss2 + se2), where ss2 is the variance component among sires and se2 is the error or residual component. Calculate the heritability estimate from the REML output and also its standard error. A heritability estimate lies between 0 and 1. Comment on the result you get.

Related reading

Allan, Eleanor and Rowlands, John 2001. Mixed Models and Multilevel Data Structures in Agriculture. International Livestock Research Institute, Nairobi, Kenya and Statistical Services Centre, The University of Reading, UK 28pp. Full text

Baker, R.L., Mwamachi, D.M., Audho, J.O., Aduda, E.O. and Thorpe, W. 1999. Gentic resistance to gastrointestinal nematode parasites in Red Maasai , Dorper and Red Maasai x Dorper ewes in the sub-humid-tropics. Animal Science 69:335-344. Abstract

Baker, R.L., Nagda, S., Rodriguez-Zas, S.L., Southey, B.R., Audho, J.O., Aduda, E.O. and Thorpe, W. 2003. Resistance and resilience to gastro-intestinal nematode parasites and relationships with productivity of Red Maasai, Dorper and Red Maasai x Dorper crossbred lambs in the sub-humid tropics. Animal Science 76:119-136. Abstract

Methu, J.N., Owen, E., Tanner, J.C. and Abate, A.L. 2001. The effect of increasing planting density and thinning on forage and grain yield of maize in Kenyan smallholdings. Abstract

Mbunzi, Stephen and Nagda, Sonal (2009). Mixed model analysis using R. Research Methods Group, ILRI, Nairobi 16pp. Full text.

Nguti, Rosemary Wangeci, 2003. Random Effects Survival Models Applied to Animal Breeding Data. Chapter 4 and 5 of PhD Thesis. Limburgs Universitair Centrum, Diepenbeek, Belgium. Full text

Nguti, R, Janssen, P., Rowlands, G.J., Audho, J.O and. Baker, R.L. 2003. Survival of Red Maasai, Dorper and crossbred lambs in the sub-humid tropics. Animal Science 76: 3-17. **Abstract**

Rege, J.E.O., Tembely, S., Mukasa-Mugerwa, E., Sovani, S., Anindo, D., Lahlou-Kassi, A. Nagda, S. and Baker, R.L. 2002. Effect of breed and season on production and response to infections with gastro-intestinal nematode parasites in sheep in the highlands of Ethiopia. Livestock Production Science 78: 159-174. Abstract

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Unless otherwise indicated the photographs were taken by Dave Elsworth