Exercise 5


```
Response: EarInfect
          Df Sum Sq Mean Sq F value    Pr(>F)
row        4  83.62  20.906  3.4428   0.04287 *
col        4  24.77   6.193  1.0199   0.43576
Treatment  4 826.56 206.640 34.0302 1.842e-06 ***
Residuals 12  72.87   6.072
```

	EarInfect	groups
Silk	48.118	a
Damage	43.518	ab
Stalk	38.952	bc
Seed	35.978	cd
Root	31.608	d

Exercise 6


```
Response: SugarYield
           Df  Sum Sq Mean Sq F value   Pr(>F)
row         3  11.778   3.926  1.0854 0.424093
col         3 101.145  33.715  9.3212 0.011231 *
Treatment   3 172.965  57.655 15.9398 0.002901 **
Residuals   6  21.702   3.617
```

```
    SugarYield groups
T0     24.3900      a
T12    21.4025     ab
T8     17.5100     bc
T4     16.0100      c
```

Linear Mixed Models

The results from a linear model are reliant on the model assumptions being met and in all examples investigated this was the case. In research there is data that do not meet these requirements, or that are not complete, or arise from more complex experiments. These data can be analysed using Linear Mixed Models (LMM) using much the same process as previously used. A LMM can be used instead of linear models, the mathematics behind the model is quite different to a linear model but the results will be equivalent.

Linear Mixed Models

A LMM has combines two models into one, a **fixed model** and a **random model**. The fixed is used to describe the explanatory structure of the experiment and the random is used to describe the structural component of the experiment.

In the first part of this chapter some of the previous examples will be revisited and analysed using a LMM approach. ASReml-R (Butler, Cullis, Gilmour, & Gogel, 2007) is used as the tool to analyse the data using the function `asreml`.

YOURTURN

LMM - Example 3 revisited

The linear mixed model that is fit can be symbolically written as:

```
Response variable  :  Yield
Structural component  :  Block
Explanatory component  :  Variety
Residual  :  Assume independence
```

Likelihood-ratio test

H_0 : `The reduced model is true or the dropped term is not significant`

H_1 : `The current model is true or the dropped term is significant`

Here to test the null hypothesis that an arbitrary group of k coefficients from the model is set equal to zero (e.g. no relationship with the response), we need to fit two **nested** models, the:

1. **reduced model** which omits the k predictors in question, and
2. **current model** which includes them.

Likelihood-ratio test

The likelihood-ratio test is

$$\Delta = -2 \times logL \texttt{ from reduced model} - (-2 \times logL \texttt{ from current model})$$

and the degrees of freedom is k (the number of coefficients in question). The p-value is $P(\chi^2_k \geq \Delta)$.
As the random terms reflect the design of the experiment, they are left in the model regardless of significance.

Exercise 7 - based on Exercise 1


```
            Df denDF  F.inc    Pr
(Intercept)  1    24 4686.0 0.000
Variety     11    24    4.7 0.001
```

	predicted.value	Variety	std.error	groups	ci	low	up
1	1.973333	Lang	0.1177883	a	0.243103	1.730230	2.216436
2	2.130000	Drysdale	0.1177883	a	0.243103	1.886897	2.373103
3	2.130000	Wylah	0.1177883	a	0.243103	1.886897	2.373103
4	2.140000	Baxter	0.1177883	a	0.243103	1.896897	2.383103
5	2.193333	Janz	0.1177883	ab	0.243103	1.950230	2.436436
6	2.240000	Endure	0.1177883	ab	0.243103	1.996897	2.483103
7	2.270000	Orion	0.1177883	ab	0.243103	2.026897	2.513103
8	2.283333	Zippy	0.1177883	ab	0.243103	2.040230	2.526436
9	2.526667	Fortune	0.1177883	ab	0.243103	2.283564	2.769770
10	2.540000	Caryina	0.1177883	ab	0.243103	2.296897	2.783103

Exercise8 - based on Exercise 2

Response: Time

	Df	denDF	F.inc	Pr
(Intercept)	1	18	1274.00	0.000
Treatment	5	18	6.46	0.001

	predicted.value	Treatment	std.error	groups	ci	low	up
1	2.1250	KC	0.1811182	a	0.3805153	1.744485	2.505515
2	2.1650	PE	0.1811182	a	0.3805153	1.784485	2.545515
3	2.6150	HL	0.1811182	ab	0.3805153	2.234485	2.995515
4	2.7700	CN	0.1811182	ab	0.3805153	2.389485	3.150515
5	2.7975	HE	0.1811182	ab	0.3805153	2.416985	3.178015
6	3.3650	CP	0.1811182	b	0.3805153	2.984485	3.745515

Exercise 9 - based on Exercise 3

Response: AverageFruitSize

	Df	denDF	F.inc	Pr
(Intercept)	1	4	65.47	0.001
Variety	6	24	25.34	0.000

	predicted.value	Treatment	std.error	groups	ci	low	up
1	2.1250	KC	0.1811182	a	0.3710039	1.753996	2.496004
2	2.1650	PE	0.1811182	a	0.3710039	1.793996	2.536004
3	2.6150	HL	0.1811182	ab	0.3710039	2.243996	2.986004
4	2.7700	CN	0.1811182	ab	0.3710039	2.398996	3.141004
5	2.7975	HE	0.1811182	ab	0.3710039	2.426496	3.168504
6	3.3650	CP	0.1811182	b	0.3710039	2.993996	3.736004

Exercise 10 - based on Exercise 4

Response: Yield

	Df	denDF	F.inc	Pr
(Intercept)	1	3	910.80	0.000
SeedingRate	5	15	1.32	0.308

Exercise 11 - based on Exercise 5

	Df	denDF	F.inc	Pr
(Intercept)	1	3.6	1868.00	0
Treatment	4	12.0	34.03	0

	predicted.value	Treatment	std.error	groups	ci	low	up
1	31.608	Root	1.346334	a	2.808404	28.7996	34.4164
2	35.978	Seed	1.346334	ab	2.808404	33.1696	38.7864
3	38.952	Stalk	1.346334	bc	2.808404	36.1436	41.7604
4	43.518	Damage	1.346334	cd	2.808404	40.7096	46.3264
5	48.118	Silk	1.346334	d	2.808404	45.3096	50.9264

Exercise 12 - based on Exercise 6

Response: SugarYield

	Df	denDF	F.inc	Pr
(Intercept)	1	3	184.90	0.001
Treatment	3	6	15.94	0.003

	predicted.value	Treatment	std.error	groups	ci	low	up
1	16.0100	T4	1.674721	a	3.648903	12.3611	19.6589
2	17.5100	T8	1.674721	ab	3.648903	13.8611	21.1589
3	21.4025	T12	1.674721	bc	3.648903	17.7536	25.0514
4	24.3900	T0	1.674721	c	3.648903	20.7411	28.0389

Split-plot Design

A split-plot design consists of blocks, that contain a complete set of treatments. Each block divided into whole plots, with levels of treatment factor A randomised to the whole plots separately with each block. Finally, each whole plot is divided into a number of subplots and the levels of factor B are randomised onto subplots within each whole plot.

Reminder

Two factors are crossed when every category of one factor co-occurs in the design with every category of the other factor. In other words, there is at least one observation in every combination of levels for the two factors.

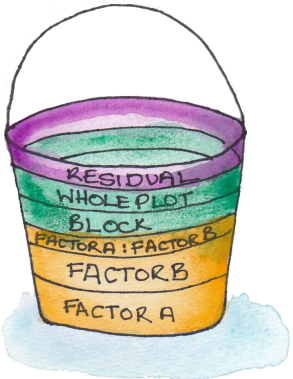
A factor is nested within another factor when each level of the first factor co-occurs with only one level of the other. In other words, an observation has to be within one level of Factor 2 in order to have a specific level of Factor 1. All combinations of levels are not represented.

If two factors are crossed, you can calculate an interaction. If they are nested, you cannot because you do not have every combination of one factor along with every combination of the other.

Split-plot Design

Source of Variation	df
Block stratum	b-1
Whole plot stratum	
Factor A	t1-1
Whole plot Residual	(t1-1)(b-1)
Subplot stratum	
Factor B	t2-1
Factor A:Factor B	(t1-1)(t2-1)
Subplot Residual	t1(t2-1)(b-1)
Total	n-1

Variance partitioning for a Split-plot design

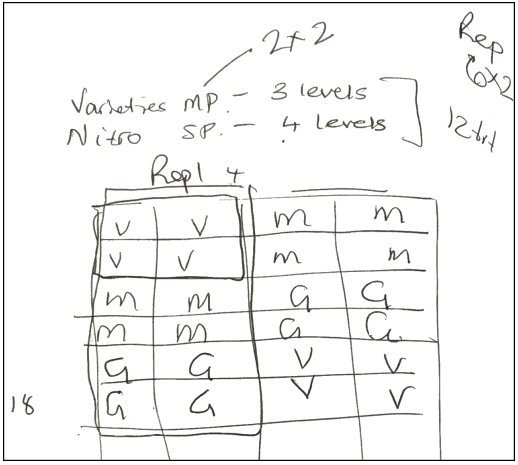


Example 5

Grown in 1995-1996 at the Scottish Crop Research Institute. Split-plot design with 4 blocks, 2 whole-plot fungicide treatments, and 70 barley varieties or variety mixes. Total area was 10 rows (north|south) by 56 columns (east|west).

It is presumed that the aim of this analysis is to determine if the treatments effect the yield, either as a combination (interaction) or as a main effect.

Experimental Layout



Split-plot

The linear mixed model that is fit can be symbolically written as:

```
Response variable  :  Yield
Structural component  :  Block, Whole-plot within Block
Explanatory component  :  Genotype, Fungicide
Residual  :  Assume independence
```

Interpreting the output

Inspection of the residual plots indicates that the model assumptions are met. Even though there are slight deviations from a true normal distribution and the Shapiro Wilks normality test indicates that the residuals are not normally distributed (p-value $<$ 0.001), the conclusion would be that the residuals approximately follow a normal distribution. LMM techniques are robust against departures from normality, so this would not be considered a serious problem in this case.
The interaction of Genotype and Fungicide is not significant, p-value ≥ 0.05.

Note: none significant interactions

In this case Genotype and Fungicide are acting independently on Yield, if this were not the case the interaction term would be significant in the model. When this happens the interaction term is removed from the model and the model is fit again. Of course the model assumptions will need to be checked again as a different model is now fitted. The main effects of the model are then assessed for significance.

Exercise 13


```
Response: Yield
             Df denDF   F.inc    Pr
(Intercept)  1     5 245.100 0.000
Genotype     2    61   3.809 0.028
Nitrogen     3    61  28.460 0.000

  predicted.value   Genotype std.error groups       ci      low       up
1         97.6250    Victory  7.152596      a 14.28062 83.34438 111.9056
2        104.5000 GoldenRain  7.152596     ab 14.28062 90.21938 118.7806
3        109.7917 Marvellous  7.152596      b 14.28062 95.51105 124.0723

  predicted.value Nitrogen std.error groups      ci       low        up
1        79.38889        0  7.339506      a 14.6538  64.73509  94.04269
2        98.88889      0.2  7.339506      b 14.6538  84.23509 113.54269
3       114.22222      0.4  7.339506      c 14.6538  99.56842 128.87602
```

Exercise 14


```
                   Df denDF  F.inc    Pr
(Intercept)         1     2 48.000 0.020
Variety             4    16  6.943 0.002
Irrigation          1     2 22.820 0.041
Variety:Irrigation  4    16  3.994 0.020

predicted.value Variety Irrigation std.error groups    ci      low       up
1      4.613333 Thumper    Rainfed 0.9902407      a 2.065606 2.547727 6.67893
2      5.470000 Cobbler    Rainfed 0.9902407     ab 2.065606 3.404394 7.53560
3      5.906667   Bravo    Rainfed 0.9902407      b 2.065606 3.841061 7.97227
4      6.193333   Hyola    Rainfed 0.9902407     bc 2.065606 4.127727 8.25893
5      6.433333 Victory    Rainfed 0.9902407     bc 2.065606 4.367727 8.49893
6      6.923333 Thumper  Irrigated 0.9902407     bc 2.065606 4.857727 8.98893
7      7.023333 Victory  Irrigated 0.9902407     bc 2.065606 4.957727 9.08893
8      7.683333   Bravo  Irrigated 0.9902407      c 2.065606 5.617727 9.74893
```

Spatial Analysis

Up until now we have seen simple linear mixed models in which the random model terms and the residual error term are all assumed to be independently and identically distributed. We call these variance component models. These models accommodate broad scale variation in the field but they do not capture small scale variation. Field trial data typically exhibit local variation (also called plot-to-plot variation or spatial trend). This can be due to moisture gradients and fertility trends in the field. This results in a higher correlation between plots that are closer to each spatially.

Spatial Analysis

In spatial analysis, broad scale variation is accommodated by including appropriate blocking factors as random terms in the analysis (for example, block in the RCB and both block and row in the LS designs). Alternatively, spatial variation is accommodated by specifying a more sophisticated spatial covariance structure for the residual error term. Spatial analysis results in estimated treatment effects that are more accurate and precise than their counterparts for more traditional methods of analysis.

Local trend reflects that data for plots that are closer together in the experimental layout are more alike than those that are further apart. As such, the residuals are correlated, with the correlation being a function of the spatial distance between the plots.

Assumption 1

It is assumed that the correlation for pairs of plots that are the same distance from each other as the crow flies and irrespective of where they are positioned in the experimental layout, is the same.

Assumption 2

Effects can be indexed by columns and rows. The distance between 2 effects is defined by their separation in the column and row direction, for example, ε_{12} and ε_{35} are 2 apart in the row direction and 3 apart in the column direction. It is assumed that the correlation between 2 smooth trend effects is the product of the correlation between the column effects of their separation in columns and the correlation between the row effects of their separation in rows. We call this the assumption of separability. The assumption of separability is computationally convenient and appears to be reasonable for the two-dimensional smooth trend process associated with field trials.

Spatial Analysis

Many forms for modelling the spatial correlation are possible. After the analysis of many hundred field trails, a separable autoregressive spatial model of order 1 (AR1 $\times$ AR1) is a plausible model for smooth trend in field trial analysis.

Rule 1

The number of effects in the residual term must be equal to the number of data units included in the analysis.

Rule 2

Where a compound model term is specified for the residuals, each combination of levels of the factors comprising this term must uniquely identify one unit of the data.

Rule 3

The data must be ordered to match the R structure specified.

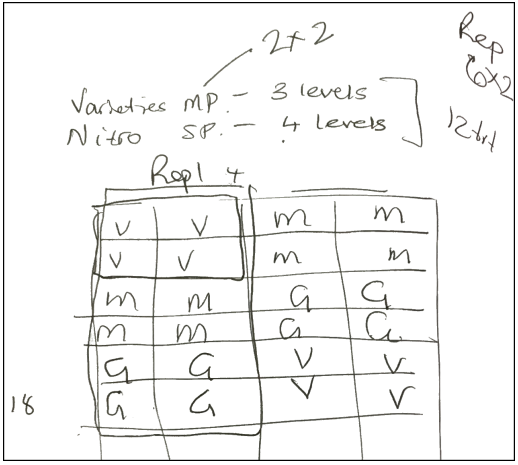
Rule 4

Never fit an autoregressive correlation structure for less than 5 rows or columns.

Example 6

A field trial to test the response of a crop to herbicide treatments was designed as a RCBD with three blocks of 21 plots. The yield at harvest was recorded for each plot. The data can be found in `example6.csv`. Is there evidence of differences in yield among the herbicide treatments?

Experimental Layout



Example 6

The linear model that is fit can be symbolically written as:

```
          Response variable  :  Yield
       Structural component  :  Block
    Explanatory component    :  Treatment
                   Residual  :  Explore spatial correlation
```

Exercise 15


```
                  Df denDF   F.inc    Pr
(Intercept)        1   5.5 247.200 0.000
Genotype           2  55.4   4.069 0.022
Nitrogen           3  42.9  45.030 0.000
Genotype:Nitrogen  6  41.4   0.465 0.830
```

```
  predicted.value   Genotype std.error groups       ci      low       up
1        97.80461    Victory  7.206781      a 14.38880 83.41580 112.1934
2       102.57666 GoldenRain  7.264648     ab 14.50434 88.07232 117.0810
3       109.27679 Marvellous  7.175646      b 14.32664 94.95015 123.6034
```

```
  predicted.value Nitrogen std.error groups       ci       low       up
1        77.08206        0  7.251809      a 14.47870  62.60335  91.56076
2        98.47013      0.2  7.209918      b 14.39506  84.07507 112.86519
3       114.44260      0.4  7.198464      c 14.37220 100.07040 128.81479
```

Exercise 16

	Df	denDF	F.inc	Pr
(Intercept)	1	12.2	273.300	0
Genotype	49	80.9	4.783	0

	predicted.value	Genotype	std.error	groups	ci	low	up
1	1.531944	G36	0.2764750	ab	0.5485185	0.983426	2.080463
2	1.590845	G28	0.2747931	ac	0.5451817	1.045663	2.136027
3	1.808187	G07	0.2701121	abce	0.5358947	1.272293	2.344082
4	1.874460	G46	0.2687801	abcef	0.5332521	1.341208	2.407712
5	1.884487	G29	0.2684802	abcef	0.5326571	1.351829	2.417144
6	1.886878	G43	0.2685921	abcef	0.5328791	1.353999	2.419757

Modelling complex treatment structures

In the design workshop we saw that it was possible to plan experiments with more than one treatment variable. We have seen the analysis of a split-plot experiment where there was a factorial treatment structure with two explanatory variables. There are other more complex treatment structures that can be used when analysing experimental data.

Modelling complex treatment structures

For example, Example 6 had 21 herbicide treatments, one of which was a control. The treatments were:

1. Achieve_250g
2. Achieve_300g
3. Achieve_380g
4. Atlantis_300mL
5. Atlantis_330mL
6. Control_0
7. Hoegrass_0.75L
8. Hoegrass_1.0L
9. Hoegrass_1.2L
10. Hussar_150g
11. Hussar_200g
12. MatavenL_2.25L
13. MatavenL_3.0L
14. Topik_50mL
15. Topik_65mL
16. Topik_85mL
17. Tristar_1.0L
18. Tristar_1.5L
19. Wildcat_250mL
20. Wildcat_300mL
21. Wildcat_350mL

Example 6

The simple hypothesis was tested:

$$H_0 \ : \ \texttt{Yield is the same for all Treatments}$$
$$H_1 \ : \ \texttt{Yield is not the same for all Treatments}$$

It can be seen that the 21 treatments are made up of 8 different herbicides with 2 or 3 different rates of application within a herbicide. This treatment structure would have been purposefully made.

Example 6

H_0 : Yield is the same for the Control and the Treatments

H_1 : Yield is not the same for the Control and the Treatments

H_0 : Yield is not affected by the different Herbicide Treatments

H_1 : Yield is affected by the different Herbicide Treatments

H_0 : Yield is not affected differently by rate of application within the Herbicide Treatments

H_1 : Yield is affected differently by rate of application within the Herbicide Treatments

Example 6

To fit a model to answer these three hypotheses the data must contain a separate columns for Control (Yes/No), Herbicide (Achieve, Atlantis, Control, Hoegrass, Hussar, MatavenL, Topik, Tristar & Wildcat) and Application Rate. Each column relates to each of the hypotheses.

	A	B	C	D	E	F	G	H	I
	Column	Row	Block	Treatment	Control	Herbicide	Rate	Yield	
	1	1	1	MatavenL_3.0L	No	MatavenL	3.0L	0.951	
	1	2	1	MatavenL_2.25L	No	MatavenL	2.25L	1.188	
	1	3	1	Hoegrass_0.75L	No	Hoegrass	0.75L	1.011	
	1	4	1	Atlantis_300mL	No	Atlantis	300mL	1.389	
	1	5	1	Achieve_300g	No	Achieve	300g	1.587	

Example 6

The linear model that is fit can be symbolically written as:

```
          Response variable  :  Yield
       Structural component  :  Block
    Explanatory component    :  Control, Herbicide, Herbicide:Rate
```

The modelling process is altered by fitting an AR1 $\times$ ID process to accommodate a smooth spatial trend.

Exercise 17

```
              Df denDF  F.inc    Pr
(Intercept)    1   4.7 64.960 0.001
Control        1  16.9 22.230 0.000
Season         1  17.9  6.816 0.018
Rate           2  16.8  4.595 0.026
```

```
  predicted.value Control std.error groups       ci      low       up
1        12.05221      No  1.871703      a 3.840417  8.21179 15.89262
2        22.84462     Yes  2.452749      b 5.032625 17.81199 27.87724

1        9.158917       F  2.132531      a 4.375593  4.783324 13.53451
2       14.945498       S  2.200014      b 4.514057 10.431441 19.45955
3       22.844618       O  2.452749      c 5.032625 17.811994 27.87724

1        7.813065     12  2.398790      a 4.921911  2.891154 12.73498
```

References

Butler, D., Cullis, B., Gilmour, A., & Gogel, B. (2007). Asreml-r reference manual [Computer software manual].