The R Book

Second Edition

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19

Mixed-Effects Models

Up to this point, we have treated all categorical explanatory variables as if they were the same. This is certainly what R.A. Fisher had in mind when he invented the analysis of variance in the 1920s and 1930s. It was Eisenhart (1947) who realized that there were actually two fundamentally different sorts of categorical explanatory variables: he called these **fixed effects** and **random effects**. It will take a good deal of practice before you are confident in deciding whether a particular categorical explanatory variable should be treated as a fixed effect or a random effect, but in essence:

- fixed effects influence only the **mean** of *y*;
- random effects influence only the **variance** of *y*.

Fixed effects are unknown constants to be estimated from the data. Random effects govern the variance–covariance structure of the response variable (see p. 519). Nesting (or hierarchical structure) of random effects is a classic source of pseudoreplication, so it important that you are able to recognize it and hence not fall into its trap. Random effects that come from the same group will be correlated, and this contravenes one of the fundamental assumptions of standard statistical models: *independence of errors*. Random effects occur in two contrasting kinds of circumstances:

- observational studies with hierarchical structure;
- designed experiments with different spatial or temporal scales.

Fixed effects have informative factor levels, while random effects often have uninformative factor levels. The distinction is best seen by an example. In most mammal species the categorical variable sex has two levels: male and female. For any individual that you find, the knowledge that it is, say, female conveys a great deal of information about the individual, and this information draws on experience gleaned from many other individuals that were female. A female will have a whole set of attributes (associated with her being female) no matter what population that individual was drawn from. Take a different categorical variable like genotype. If we have two genotypes in a population we might label them A and B. If we take two more genotypes from a *different* population we might label them A and B as well. In a case like this, the label A does not convey any information at all about the genotype, other than that it is probably different from genotype B. In the case of sex, the factor level (male or female) is informative: sex is a fixed effect. In the case of genotype, the factor level (A or B) is uninformative: genotype is a random effect.

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Random effects have factor levels that are drawn from a large (potentially very large) population in which the individuals differ in many ways, but we do not know exactly how or why they differ. To get a feel for the difference between fixed effects and random effects here are some more examples:

Fixed effects	Random effects
Drug administered or not	Genotype
Insecticide sprayed or not	Brood
Nutrient added or not	Block within a field
One country versus another	Split plot within a plot
Male or female	History of development
Upland or lowland	Household
Wet versus dry	Individuals with repeated measures
Light versus shade	Family
One age versus another	Parent

The important point is that because the random effects come from a large population, there is not much point in concentrating on estimating means of our small subset of factor levels, and no point at all in comparing individual pairs of means for different factor levels. Much better to recognize them for what they are, random samples from a much larger population, and to concentrate on their variance. This is the *added* variation caused by differences between the levels of the random effects.

Variance components analysis is all about estimating the size of this variance, and working out its percentage contribution to the overall variation. There are five fundamental assumptions of linear mixed-effects models:

- Within-group errors are independent with mean zero and variance σ^2.
- Within-group errors are independent of the random effects.
- The random effects are normally distributed with mean zero and covariance matrix Ψ.
- The random effects are independent in different groups.
- The covariance matrix does not depend on the group.

The validity of these assumptions needs to be tested by employing a series of plotting methods involving the residuals, the fitted values and the predicted random effects. The tricks with mixed-effects models are:

- learning which variables are random effects;
- specifying the fixed and random effects in the model formula;
- getting the nesting structure of the random effects right;
- remembering to get `library(lme4)` or `library(nlme)` at the outset.

The issues fall into two broad categories: questions about experimental design and the management of experimental error (e.g. where does most of the variation occur, and where would increased replication be most profitable?); and questions about hierarchical structure, and the relative magnitude of variation at different levels within the hierarchy (e.g. studies on the genetics of individuals within families, families within parishes, and parishes with counties, to discover the relative importance of genetic and phenotypic variation).

Most ANOVA models are based on the assumption that there is a single error term. But in hierarchical studies and nested experiments, where the data are gathered at two or more different spatial scales, there is *a different error variance for each different spatial scale*. There are two reasonably clear-cut sets of circumstances where your first choice would be to use a linear mixed-effects model: you want to do variance components analysis because all your explanatory variables are categorical random effects and you do not have any fixed effects; or you do have fixed effects, but you also have pseudoreplication of one sort or another (e.g. temporal pseudoreplication resulting from repeated measurements on the same individuals; see p. 699). To test whether one should use a model with mixed effects or just a plain old linear model, Douglas Bates wrote in the R help archive: 'I would recommend the likelihood ratio test against a linear model fit by `lm`. The *p*-value returned from this test will be conservative because you are testing on the boundary of the parameter space.'

19.1 Replication and pseudoreplication

To qualify as replicates, measurements must have the following properties:

- They must be independent.
- They must not form part of a time series (data collected from the same place on successive occasions are not independent).
- They must not be grouped together in one place (aggregating the replicates means that they are not spatially independent).
- They must be of an appropriate spatial scale;
- Ideally, one replicate from each treatment ought to be grouped together into a block, and the whole experiment repeated in many different blocks.
- Repeated measures (e.g. from the same individual or the same spatial location) are not replicates (this is probably the commonest cause of pseudoreplication in statistical work).

Pseudoreplication occurs when you analyse the data as if you had more degrees of freedom than you really have. There are two kinds of pseudoreplication:

- temporal pseudoreplication, involving repeated measurements from the same individual;
- spatial pseudoreplication, involving several measurements taken from the same vicinity.

Pseudoreplication is a problem because one of the most important assumptions of standard statistical analysis is *independence of errors*. Repeated measures through time on the same individual will have non-independent errors because peculiarities of the individual will be reflected in all of the measurements made on it (the repeated measures will be temporally correlated with one another). Samples taken from the same vicinity will have non-independent errors because peculiarities of the location will be common to all the samples (e.g. yields will all be high in a good patch and all be low in a bad patch).

Pseudoreplication is generally quite easy to spot. The question to ask is this. How many degrees of freedom for error does the experiment really have? If a field experiment appears to have lots of degrees of freedom, it is probably pseudoreplicated. Take an example from pest control of insects on plants. There are 20 plots, 10 sprayed and 10 unsprayed. Within each plot there are 50 plants. Each plant is measured five times during

the growing season. Now this experiment generates $20 \times 50 \times 5 = 5000$ numbers. There are two spraying treatments, so there must be 1 degree of freedom for spraying and 4998 degrees of freedom for error. Or must there? Count up the replicates in this experiment. Repeated measurements on the same plants (the five sampling occasions) are certainly not replicates. The 50 individual plants within each quadrat are not replicates either. The reason for this is that conditions within each quadrat are quite likely to be unique, and so all 50 plants will experience more or less the same unique set of conditions, irrespective of the spraying treatment they receive. In fact, there are 10 replicates in this experiment. There are 10 sprayed plots and 10 unsprayed plots, and each plot will yield only one independent datum for the response variable (the mean proportion of leaf area consumed by insects, for example). Thus, there are 9 degrees of freedom within each treatment, and $2 \times 9 = 18$ degrees of freedom for error in the experiment as a whole. It is not difficult to find examples of pseudoreplication on this scale in the literature (Hurlbert 1984). The problem is that it leads to the reporting of masses of spuriously significant results (with 4998 degrees of freedom for error, it is almost impossible *not* to have significant differences). The first skill to be acquired by the budding experimenter is the ability to plan an experiment that is properly replicated. There are various things that you can do when your data are pseudoreplicated:

- Average away the pseudoreplication and carry out your statistical analysis on the means.
- Carry out separate analyses for each time period.
- Use proper time series analysis or mixed-effects models.

19.2 The `lme` and `lmer` functions

Most of the examples in this chapter use the linear mixed model formula `lme`. This is to provide compatibility with the excellent book by Pinheiro and Bates (2000) on *Mixed-Effects Models in S and S-PLUS*. More recently, however, Douglas Bates has released the generalized mixed model function `lmer` as part of the `lme4` package, and you may prefer to use this in your own work, especially for nested count data or proportion data. To begin with, I provide a simple comparison of the basic syntax of the two functions.

19.2.1 `lme`

Specifying the fixed and random effects in the model formula is done with two formulae. Suppose that there are no fixed effects, so that all of the categorical variables are random effects. Then the fixed effect simply estimates the intercept (parameter 1):

```
fixed = y ~ 1
```

The fixed effect (a compulsory part of the `lme` structure) is just the overall mean value of the response variable `y ~ 1`. The `fixed =` part of the formula is optional if you put this object first. The random effects show the identities of the random variables and their relative locations in the hierarchy. The three random effects (`a`, `b`, and `c`) are specified like this:

```
random = ~ 1 | a/b/c
```

and in this case the phrase `random =` is *not* optional. An important detail to notice is that the name of the response variable (`y`) is not repeated in the random-effects formula: there is a blank space to the left of the tilde `~`. In most mixed-effects models we assume that the random effects have a mean of zero and that we are interested in quantifying variation in the intercept caused by differences between the factor levels of the

random effects. After the intercept comes the vertical bar | which is read as 'given the following spatial arrangement of the random variables'. In this example there are three random effects with 'c nested within b which in turn is nested within a'. The factors are separated by forward slash characters, and the variables are listed from left to right in declining order of spatial (or temporal) scale. This will only become clear with practice, but it is a simple idea. The formulae are put together like this:

```
lme(fixed = y ~ 1, random = ~ 1 | a/b/c)
```

19.2.2 lmer

There is just one formula in lmer, not separate formulae for the fixed and random effects. The fixed effects are specified first, to the right of the tilde, in the normal way. Next comes a plus sign, then one or more random terms enclosed in parentheses (in this example there is just one random term, but we might want separate random terms for the intercept and for the slopes, for instance). R can identify the random terms because they must contain a 'given' symbol |, to the right of which are listed the random effects in the usual way, from largest to smallest scale, left to right. So the lmer formula for this example is:

```
lmer(y ~ 1+( 1 | a/b/c ))
```

19.3 Best linear unbiased predictors

In aov, the effect size for treatment i is defined as $\bar{y}_i - \mu$, where μ is the overall mean. In mixed-effects models, however, correlation between the pseudoreplicates within a group causes what is called **shrinkage**. The best linear unbiased predictors (BLUPs, denoted by a_i) are smaller than the effect sizes ($\bar{y}_i - \mu$), and are given by

$$a_i = (\bar{y}_i - \mu)\left(\frac{\sigma_a^2}{\sigma_a^2 + \sigma^2/n}\right),$$

where σ^2 is the residual variance and σ_a^2 is the between-group variance which introduces the correlation between the pseudoreplicates within each group. Thus, the parameter estimate a_i is 'shrunk' compared to the fixed effect size ($\bar{y}_i - \mu$). When σ_a^2 is estimated to be large compared with the estimate of σ^2/n (i.e. when most of the variation is between classes and there is little variation within classes), the fixed effects and the BLUP are similar. On the other hand, when σ_a^2 is estimated to be small compared with the estimate of σ^2/n, then the fixed effects and the BLUP can be very different.

19.4 Designed experiments with different spatial scales: Split plots

The important distinction in models with categorical explanatory variables is between cases where the data come from a designed experiment, in which treatments were allocated to locations or subjects at random, and cases where the data come from an observational study in which the categorical variables are associated with an observation before the study. Here, we call the first case split-plot experiments and the second case hierarchical designs. The point is that their dataframes look identical, so it is easy to analyse one case wrongly as if it were the other. You need to be able to distinguish between fixed effects and random effects in both cases.

Here is the linear model for a split-plot experiment analysed in Chapter 11 by `aov` (see p. 519):

```
yields <- read.table("c:\\temp\\splityield.txt",header=T)
attach(yields)
names(yields)
```

```
[1] "yield" "block" "irrigation" "density" "fertilizer"
```

```
library(nlme)
```

The fixed-effects part of the model is specified in just the same way as in a straightforward factorial experiment: `yield ~ irrigation*density*fertilizer`. The random-effects part of the model says that we want the random variation to enter via effects on the intercept as `random=~1`. Finally, we define the spatial structure of the random effects after the 'given' symbol | as: `block/irrigation/density` reflecting the progressively smaller plot sizes. There is no need to specify the smallest spatial scale (fertilizer plots in this example).

```
model <- lme(yield ~ irrigation*density*fertilizer,random= ~
1|block/irrigation/density)
summary(model)
```

```
Linear mixed-effects model fit by REML
 Data: NULL
       AIC       BIC    logLik
  481.6212  525.3789 -218.8106

Random effects:
 Formula: ~1 | block
        (Intercept)
StdDev: 0.000660972

 Formula: ~1 | irrigation %in% block
        (Intercept)
StdDev:    1.982463

 Formula: ~1 | density %in% irrigation %in% block
        (Intercept) Residual
StdDev:    6.975553 9.292805

Fixed effects: yield ~ irrigation * density * fertilizer
                                              Value Std.Error DF   t-value p-value
(Intercept)                                   80.50  5.893741 36 13.658558  0.0000
irrigationirrigated                           31.75  8.335008  3  3.809234  0.0318
densitylow                                     5.50  8.216281 12  0.669403  0.5159
densitymedium                                 14.75  8.216281 12  1.795216  0.0978
fertilizerNP                                   5.50  6.571005 36  0.837010  0.4081
fertilizerP                                    4.50  6.571005 36  0.684827  0.4978
irrigationirrigated:densitylow               -39.00 11.619577 12 -3.356405  0.0057
irrigationirrigated:densitymedium            -22.25 11.619577 12 -1.914872  0.0796
irrigationirrigated:fertilizerNP              13.00  9.292805 36  1.398932  0.1704
irrigationirrigated:fertilizerP                5.50  9.292805 36  0.591856  0.5576
densitylow:fertilizerNP                        3.25  9.292805 36  0.349733  0.7286
densitymedium:fertilizerNP                    -6.75  9.292805 36 -0.726368  0.4723
densitylow:fertilizerP                        -5.25  9.292805 36 -0.564953  0.5756
densitymedium:fertilizerP                     -5.50  9.292805 36 -0.591856  0.5576
irrigationirrigated:densitylow:fertilizerNP    7.75 13.142011 36  0.589712  0.5591
irrigationirrigated:densitymedium:fertilizerNP 3.75 13.142011 36  0.285344  0.7770
irrigationirrigated:densitylow:fertilizerP     20.00 13.142011 36  1.521837  0.1368
irrigationirrigated:densitymedium:fertilizerP   4.00 13.142011 36  0.304367  0.7626
```

This output suggests that the only significant effects are the main effect of `irrigation` ($p = 0.0318$) and the `irrigation` by `density` interaction ($p = 0.0057$). The three-way interaction is not significant so we remove it, fitting all terms up to two-way interactions:

```
model <-  lme(yield~(irrigation+density+fertilizer)^2,
                        random=~1|block/irrigation/density)
summary(model)
```

```
Linear mixed-effects model fit by REML
 Data: NULL
       AIC      BIC    logLik
  503.1256 540.2136 -233.5628

Random effects:
 Formula: ~1 | block
         (Intercept)
StdDev: 0.0005634512

 Formula: ~1 | irrigation %in% block
        (Intercept)
StdDev:    1.982562

 Formula: ~1 | density %in% irrigation %in% block
        (Intercept) Residual
StdDev:    7.041303 9.142696

Fixed effects: yield ~ (irrigation + density + fertilizer)^2
                                    Value Std.Error DF   t-value p-value
(Intercept)                      82.47222  5.443438 40 15.150760  0.0000
irrigationirrigated              27.80556  7.069256  3  3.933307  0.0293
densitylow                        0.87500  7.256234 12  0.120586  0.9060
densitymedium                    13.45833  7.256234 12  1.854727  0.0884
fertilizerNP                      3.58333  5.278538 40  0.678850  0.5011
fertilizerP                       0.50000  5.278538 40  0.094723  0.9250
irrigationirrigated:densitylow  -29.75000  8.800165 12 -3.380618  0.0055
irrigationirrigated:densitymedium -19.66667  8.800165 12 -2.234807  0.0452
irrigationirrigated:fertilizerNP  16.83333  5.278538 40  3.189014  0.0028
irrigationirrigated:fertilizerP   13.50000  5.278538 40  2.557526  0.0144
densitylow:fertilizerNP           7.12500  6.464862 40  1.102112  0.2770
densitymedium:fertilizerNP       -4.87500  6.464862 40 -0.754076  0.4552
densitylow:fertilizerP            4.75000  6.464862 40  0.734741  0.4668
densitymedium:fertilizerP        -3.50000  6.464862 40 -0.541388  0.5912
```

The `fertilizer` by `density` interaction is not significant, so we remove it:

```
model <- lme(yield~irrigation*density+irrigation*fertilizer,
                              random=~1|block/irrigation/density)
summary(model)
```

```
Linear mixed-effects model fit by REML
Data: NULL
      AIC      BIC    logLik
 519.9035 549.6834 -245.9517

Random effects:
 Formula: ~1 | block
```

```
         (Intercept)
StdDev: 0.0005566885

 Formula: ~1 | irrigation %in% block
        (Intercept)
StdDev:    1.982614

 Formula: ~1 | density %in% irrigation %in% block
        (Intercept) Residual
StdDev:    7.057132 9.105995

Fixed effects: yield ~ irrigation * density + irrigation * fertilizer
                                      Value Std.Error DF   t-value p-value
(Intercept)                        82.08333  4.994999 44 16.433103  0.0000
irrigationirrigated                27.80556  7.063995  3  3.936236  0.0292
densitylow                          4.83333  6.222653 12  0.776732  0.4524
densitymedium                      10.66667  6.222653 12  1.714167  0.1122
fertilizerNP                        4.33333  3.717507 44  1.165656  0.2500
fertilizerP                         0.91667  3.717507 44  0.246581  0.8064
irrigationirrigated:densitylow    -29.75000  8.800161 12 -3.380620  0.0055
irrigationirrigated:densitymedium -19.66667  8.800161 12 -2.234808  0.0452
irrigationirrigated:fertilizerNP   16.83333  5.257349 44  3.201867  0.0025
irrigationirrigated:fertilizerP    13.50000  5.257349 44  2.567834  0.0137
```

Both the `irrigation` by `fertilizer` and `irrigation` by `density` interactions are now highly significant. The apparently non-significant main effect of `density` is spurious because `density` appears in a significant interaction with `irrigation`. The moral is that you must do the model simplification to get the appropriate *p* values.

Remember, too, that if you want to use `anova` to compare mixed models with different fixed-effects structures, then you must use maximum likelihood (`method = "ML"` in `lme` but `REML = FALSE` in `lmer`) rather than the default restricted maximum likelihood (REML). Here is the analysis again, but this time using `anova` to compare models with progressively simplified fixed effects:

```
model.lme <- lme(yield~irrigation*density*fertilizer,
                 random=~ 1| block/irrigation/density,method="ML")
model.lme.2 <- update(model.lme,~. - irrigation:density:fertilizer)
anova(model.lme,model.lme.2)
```

```
           Model df      AIC      BIC    logLik   Test  L.Ratio p-value
model.lme      1 22 573.5108 623.5974 -264.7554
model.lme.2    2 18 569.0046 609.9845 -266.5023 1 vs 2 3.493788  0.4788
```

```
model.lme.3 <- update(model.lme.2,~. - density:fertilizer)
anova(model.lme.3,model.lme.2)
```

```
           Model df      AIC      BIC    logLik   Test  L.Ratio p-value
model.lme.3    1 14 565.1933 597.0667 -268.5967
model.lme.2    2 18 569.0046 609.9845 -266.5023 1 vs 2 4.188774  0.3811
```

```
model.lme.4 <- update(model.lme.3,~. - irrigation:fertilizer)
anova(model.lme.3,model.lme.4)
```

```
            Model df      AIC      BIC    logLik   Test  L.Ratio p-value
model.lme.3     1 14 565.1933 597.0667 -268.5967
model.lme.4     2 12 572.3373 599.6573 -274.1687 1 vs 2 11.14397  0.0038
```

```
model.lme.5 <- update(model.lme.2,~. - irrigation:density)
anova(model.lme.5,model.lme.2)
```

```
            Model df      AIC      BIC    logLik   Test  L.Ratio p-value
model.lme.5     1 16 576.7134 613.1400 -272.3567
model.lme.2     2 18 569.0046 609.9845 -266.5023 1 vs 2 11.70883  0.0029
```

The `irrigation` by `fertilizer` interaction is more significant ($p = 0.0038$ compared to $p = 0.0081$) under this mixed-effects model than it was in the linear model earlier, as is the `irrigation` by `density` interaction ($p = 0.0029$ compared to $p = 0.01633$). You need to do the model simplification in `lme` to uncover the significance of the main effect and interaction terms, but it is worth it, because the `lme` analysis can be more powerful. The minimal adequate model under the `lme` is:

```
summary(model.lme.3)
```

```
Linear mixed-effects model fit by maximum likelihood
 Data: NULL
       AIC      BIC    logLik
  565.1933 597.0667 -268.5967

Random effects:
 Formula: ~1 | block
        (Intercept)
StdDev: 0.0005260885

 Formula: ~1 | irrigation %in% block
      (Intercept)
StdDev:    1.716888

 Formula: ~1 | density %in% irrigation %in% block
        (Intercept) Residual
StdDev:    5.722413 8.718327

Fixed effects: yield ~ irrigation + density + fertilizer
                       + irrigation:density + irrigation:fertilizer

                                   Value Std.Error DF   t-value p-value
(Intercept)                    82.08333  4.756285 44 17.257867  0.0000
irrigationirrigated            27.80556  6.726403  3  4.133793  0.0257
densitylow                      4.83333  5.807347 12  0.832279  0.4215
densitymedium                  10.66667  5.807347 12  1.836754  0.0911
fertilizerNP                    4.33333  3.835552 44  1.129781  0.2647
fertilizerP                     0.91667  3.835552 44  0.238992  0.8122
irrigationirrigated:densitylow -29.75000  8.212829 12 -3.622382  0.0035
irrigationirrigated:densitymedium -19.66667  8.212829 12 -2.394628  0.0338
irrigationirrigated:fertilizerNP  16.83333  5.424290 44  3.103325  0.0033
irrigationirrigated:fertilizerP   13.50000  5.424290 44  2.488805  0.0167
```

You should pay special attention to the degrees of freedom column. Note that the degrees of freedom are not pseudoreplicated: there are only 3 d.f. for testing the `irrigation` main effect; 12 d.f. for testing the `irrigation` by `density` interaction and 44 d.f. for `irrigation` by `fertilizer` (this is 36 + 4 + 4 = 44 after model simplification). Also, remember that you must do your model simplification using maximum likelihood (`method = "ML"`) because you cannot use `anova` to compare models with different fixed-effect structures using REML.

Model-checking plots show that the residuals are well behaved:

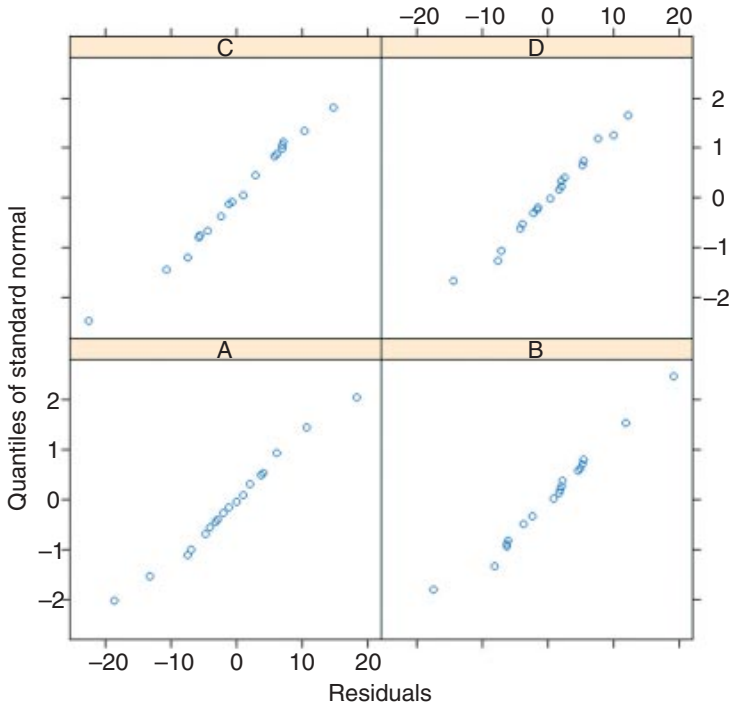
```
plot(model.lme.3)
```

the response variable is a reasonably linear function of the fitted values:

```
plot(model.lme.3,yield~fitted(.))
```

and the errors are reasonably close to normally distributed in all four blocks:

```
qqnorm(model.lme.3,~ resid(.)| block)
```



When, as here, the experiment is balanced and there are no missing values, then it is much simpler to interpret the `aov` using an `Error` term to describe the structure of the spatial pseudoreplication (p. 526), not least because it produces separate ANOVA tables for each of the spatial scales, which makes it very easy to check that these is no pseudoreplication. Without balance, however, you will need to use `lme` or `lmer` and to use model simplification to estimate the *p* values of the significant interaction terms.

If you do this example using `lmer`, you will want to switch off the matrix of correlations for the fixed effects. You do this with the `print(model,cor=F)` option (rather than `summary`):

```
library(lme4)

b <- block
bi <- block:irrigation
```

```
bid <- block:irrigation:density

model1 <-
lmer(yield~irrigation*density*fertilizer+(1|b)+(1|bi)+(1|bid),REML=FALSE)

print(model1,cor=F)
```

```
Linear mixed model fit by maximum likelihood
Formula: yield ~ irrigation * density * fertilizer + (1|b) + (1|bi) + (1|bid)
         AIC   BIC logLik deviance REMLdev
       573.5 623.6 -264.8    529.5   437.6
Random effects:
Groups   Name        Variance   Std.Dev.
bid      (Intercept) 3.6493e+01 6.0410e+00
bi       (Intercept) 2.9479e+00 1.7169e+00
b        (Intercept) 8.9145e-13 9.4417e-07
Residual             6.4767e+01 8.0478e+00
Number of obs: 72, groups: bid, 24; bi, 8; b, 4
Fixed effects:
                                            Estimate Std. Error t value
(Intercept)                                   80.500      5.104  15.772
irrigationirrigated                           31.750      7.218   4.399
densitylow                                     5.500      7.115   0.773
densitymedium                                 14.750      7.115   2.073
fertilizerNP                                   5.500      5.691   0.966
fertilizerP                                    4.500      5.691   0.791
irrigationirrigated:densitylow               -39.000     10.063  -3.876
irrigationirrigated:densitymedium            -22.250     10.063  -2.211
irrigationirrigated:fertilizerNP              13.000      8.048   1.615
irrigationirrigated:fertilizerP                5.500      8.048   0.683
densitylow:fertilizerNP                        3.250      8.048   0.404
densitymedium:fertilizerNP                    -6.750      8.048  -0.839
densitylow:fertilizerP                        -5.250      8.048  -0.652
densitymedium:fertilizerP                     -5.500      8.048  -0.683
irrigationirrigated:densitylow:fertilizerNP    7.750     11.381   0.681
irrigationirrigated:densitymedium:fertilizerNP 3.750     11.381   0.329
irrigationirrigated:densitylow:fertilizerP    20.000     11.381   1.757
irrigationirrigated:densitymedium:fertilizerP  4.000     11.381   0.351
```

As before, it requires model simplification before the significant interactions become evident.

19.5 Hierarchical sampling and variance components analysis

Hierarchical data are often encountered in observational studies where information is collected at a range of different spatial scales. The principal aim is to discover the scale at which most of the variation is generated. This information would then allow a closer focus on mechanisms operating at this scale in subsequent more detailed studies. The following study involves a test with a mean score of 100 administered to children in four British towns. Each town was divided into districts by postcodes, and six districts were selected at random. Within districts, 10 streets were selected at random, and within streets, four households were

selected at random. Naturally, different households had different numbers of children (childless households were excluded from the study) and there was no control over sex ratio of children within household.

```
library(lme4)
```

```
data <- read.table("c:\\temp\\childfull.txt",header=T)
attach(data)
head(data)
```

```
  childID child house street district  town response gender
1       1     1 door1      1        A Leeds 83.88773   male
2       1     1 door2      1        A Leeds 99.96294   male
3       1     3 door3      1        A Leeds 87.20253 female
4       2     3 door3      1        A Leeds 89.37665   male
5       3     3 door3      1        A Leeds 92.01751 female
6       1     5 door4      1        A Leeds 87.12672 female
```

You can see that the factor levels are not unique: for instance, there is a street 1 in each district of Leeds (and of every other town). We use the colon operator to create unique factor levels for each random effect: district within town (`d`), street within district within town (`s`) and household within street within district within town (`h`). Each household has one or more children (maximum = 8, mostly in Leeds) but the sex ratio varies from house to house.

```
d <- town:district
s <- town:district:factor(street)
h <- town:district:factor(street):house
```

The mixed effects model has one fixed effect (gender) and four nested random effects:

```
model <- lmer(response~gender+(1|town)+(1|d)+(1|s)+(1|h))
summary(model)
```

```
Linear mixed model fit by REML
Formula: response ~ gender + (1 | town) + (1 | d) + (1 | s) + (1 | h)
   AIC   BIC logLik deviance REMLdev
 19878 19920  -9932    19868   19864
Random effects:
Groups   Name        Variance Std.Dev.
h        (Intercept)   4.0817  2.0203
s        (Intercept)  15.6747  3.9591
d        (Intercept) 168.3451 12.9748
town     (Intercept)  36.9802  6.0811
Residual              36.2405  6.0200
```

```
Number of obs: 2972, groups: h, 960; s, 240; d, 24; town, 4
```

```
Fixed effects:
            Estimate Std. Error t value
(Intercept)  97.8965     4.0424  24.218
gendermale    0.5368     0.2363   2.272
```

```
Correlation of Fixed Effects:
           (Intr)
gendermale -0.030
```

The fixed effect is significant ($t = 2.272$) but small (0.537) compared to the means for towns, which varied by as much as 20 (e.g. Coventry vs. Derby). Of the random effects, most of the variation in the response is between districts within towns (64%) and least is between households within streets (4%). You get the percentage variance components like this:

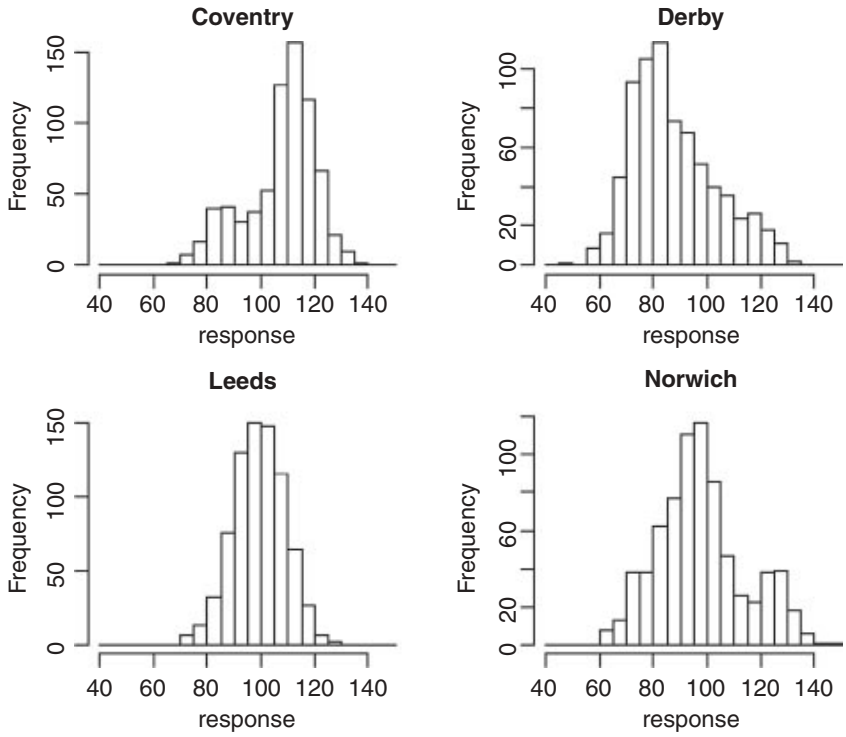
```
vc <- c(36.2405,4.0817,15.6747,168.3451,36.9802)
vc <- 100*c(36.2405,4.0817,15.6747,168.3451,36.9802)/sum(vc)
vc
```

```
[1] 13.868129  1.561942  5.998227 64.420512 14.151190
```

The key feature of these data, however, is the substantial variation between children within the same family (i.e. even after you have controlled for family and environment (town and district within town)).

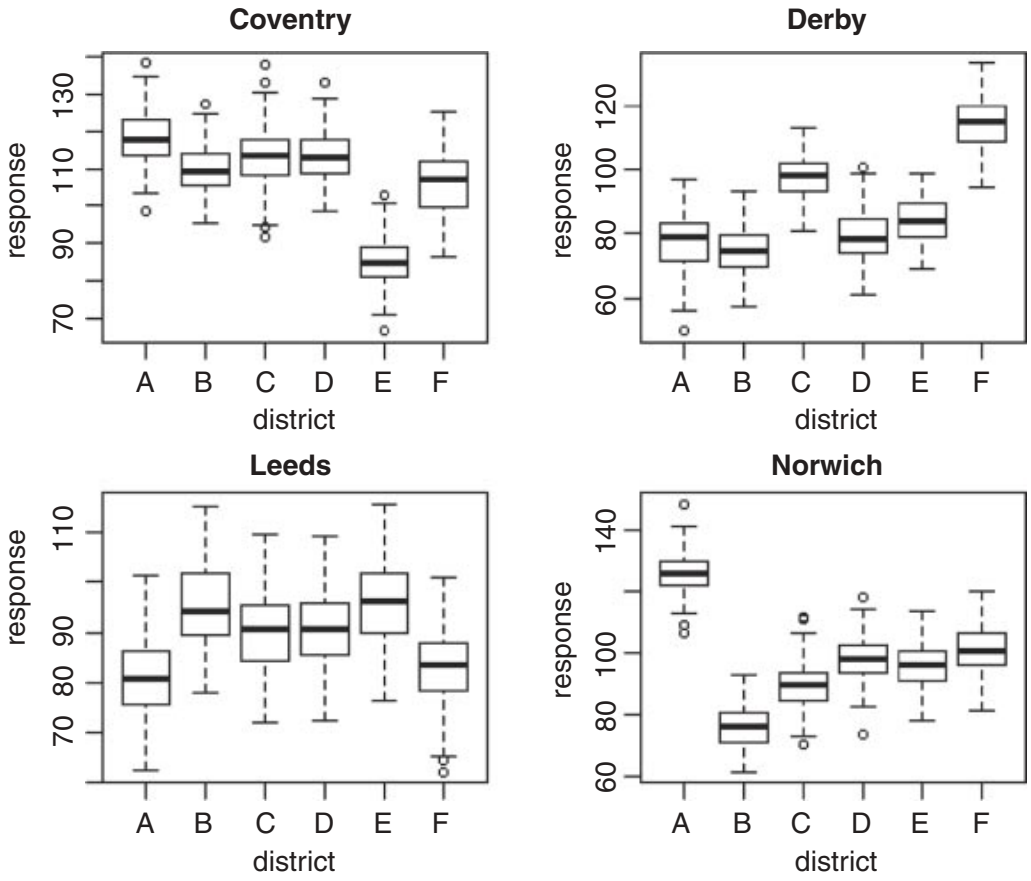
There are issues about the appropriate graphics in studies like this, simply because there are so many combinations of factor levels, and the nested factor levels typically make sense only in the context of their higher-level associations. Here is one way of showing the variation:

```
par(mfrow=c(2,2))
hist(response[town=="Coventry"],main="Coventry",breaks=seq(40,150,5),
      xlab="response")
hist(response[town=="Derby"],main="Derby",breaks=seq(40,150,5),
      xlab="response")
hist(response[town=="Leeds"],main="Leeds",breaks=seq(40,150,5),
      xlab="response")
hist(response[town=="Norwich"],main="Norwich",breaks=seq(40,150,5),
      xlab="response")
```



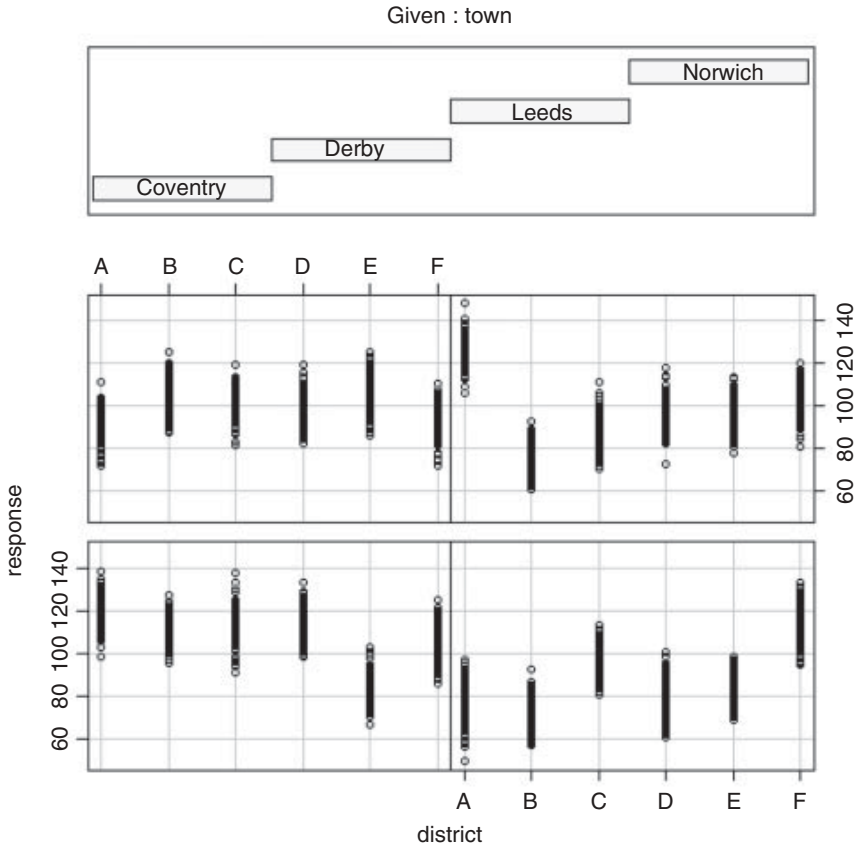
This is good at highlighting non-normality (e.g. the bimodal distribution of the response in Norwich, and the opposite skew in Coventry and Derby). Here is an alternative using box-and-whisker plots, with `subset` to chose the towns rather than subscripts:

```
plot(response~district,subset=(town=="Coventry"),main="Coventry")
plot(response~district,subset=(town=="Derby"),main="Derby")
plot(response~district,subset=(town=="Leeds"),main="Leeds")
plot(response~district,subset=(town=="Norwich"),main="Norwich")
```



This is particularly good for drawing attention to districts with strikingly different mean scores (e.g. high ones like district A in Norwich and low ones like district E in Coventry). For looking at interactions, `coplot` is often useful:

```
coplot(response~district|town)
```



In practice, you will want to try many different kinds of plots across all of the spatial scales.

19.6 Mixed-effects models with temporal pseudoreplication

A common cause of temporal pseudoreplication in growth experiments with fixed effects is when each individual is measured several times as it grows during the course of an experiment. The next example is as simple as possible: we have a single fixed effect (a two-level categorical variable, with fertilizer added or not) and six replicate plants in each treatment, with each plant measured on five occasions (after 2, 4, 6, 8 or 10 weeks of growth). The response variable is root length, measured non-destructively through a glass panel, which is opened to the light only when the root length measurements are being taken. The fixed-effect formula looks like this:

```
fixed = root~fertilizer
```

The random-effects formula needs to indicate that the week of measurement (a continuous random effect) represents pseudoreplication within each individual plant:

```
random = ~week|plant
```

Because we have a continuous random effect (weeks) we write `~week` in the random-effects formula rather than the `~1` that we used with categorical random effects (above). Here are the data:

```
results <- read.table("c:\\temp\\fertilizer.txt",header=T)
attach(results)
```

```
names(results)
```

```
[1]  "root"  "week"  "plant"  "fertilizer"
```

We begin with data inspection. For the kind of data involved in mixed-effects models there are some excellent built-in plotting functions (variously called panel plots, trellis plots, or lattice plots).

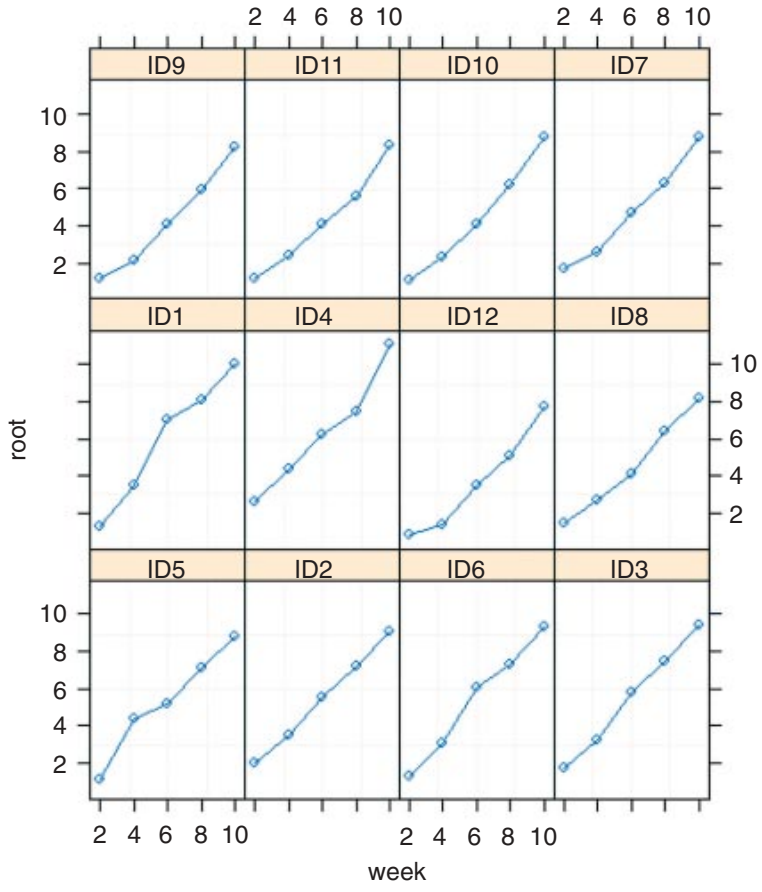
```
library(nlme)
library(lattice)
```

To use trellis plotting, we begin by turning our dataframe called `results` into a `groupedData` object (p. 957). To do this we specify the nesting structure of the random effects, and indicate the fixed effect by defining `fertilizer` as `outer` to this nesting (a fixed effect):

```
results <- groupedData(root~week|plant,outer = ~ fertilizer,results)
```

Because results is now a `groupedData` object, the plotting is fantastically simple:

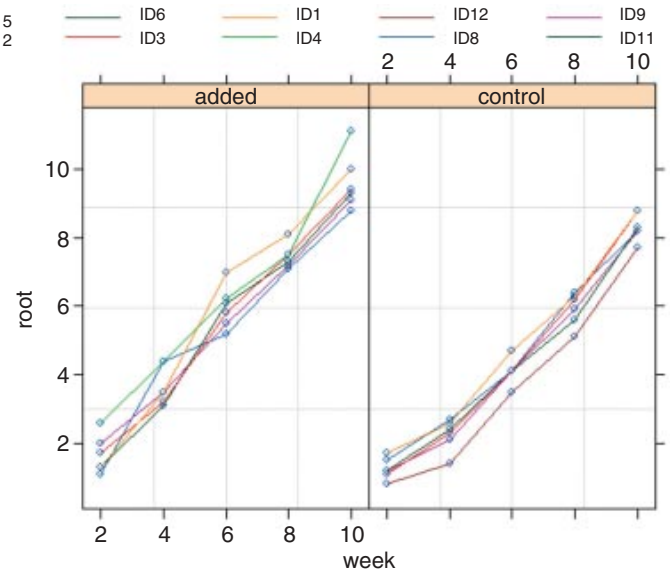
```
plot(results)
```



Here you get separate time series plots for each of the individual plants (created, in this case, by joining the dots, which is the default option), with plant identities ranked from bottom left (ID5) to top right (ID7) on the basis of mean root length. In terms of understanding the fixed effects, it is often more informative to group together the six replicates within each treatment, and to have one panel for each of the treatment levels (i.e.

one for the fertilized plants and one for the controls in this case). This is very straightforward, using `outer` to indicate the grouping:

```
plot(results,outer=T)
```



You can see that by week 10 there is virtually no overlap between the two treatment groups. The largest control plant has about the same root length as the smallest fertilized plant (about 9 cm).

Now for the statistical modelling. Ignoring the pseudoreplication, we should have 1 d.f. for `fertilizer` and $2 \times (6-1) = 10$ d.f. for error.

```
model <- lme(root~fertilizer,random=~week|plant)
summary(model)
```

```
Linear mixed-effects model fit by REML
 Data: NULL
       AIC      BIC    logLik
  171.0236 183.3863 -79.51181

Random effects:
 Formula: ~week | plant
 Structure: General positive-definite, Log-Cholesky parametrization
            StdDev    Corr
(Intercept) 2.8639832 (Intr)
week        0.9369412 -0.999
Residual    0.4966308

Fixed effects: root ~ fertilizer
                    Value Std.Error DF   t-value p-value
(Intercept)         2.799710 0.1438367 48 19.464499   0e+00
fertilizercontrol -1.039383 0.2034158 10 -5.109645   5e-04
 Correlation:
                  (Intr)
fertilizercontrol -0.707
```

```
Standardized Within-Group Residuals:
       Min         Q1        Med         Q3        Max
-1.9928118 -0.6586834 -0.1004301  0.6949714  2.0225381

Number of Observations: 60
Number of Groups: 12
```

The output looks dauntingly complex, but once you learn your way around it, the essential information is relatively easy to extract. The mean reduction in root size associated with the unfertilized controls is –1.039 383 and this has a standard error of 0.203 415 8 based on the correct 10 residual d.f. (six replicates per factor level). Can you see why the intercept has 48 d.f.? (Hint: ask yourself how many graphs have been fitted to the data.)

Here is a simple one-way ANOVA for the non-pseudoreplicated data taken from the end of the experiment in week 10:

```
model2 <- aov(root~fertilizer,subset=(week==10))
summary(model2)
```

```
            Df Sum Sq Mean Sq F value Pr(>F)
fertilizer   1  4.941   4.941   11.49 0.0069 **
Residuals   10  4.302   0.430
```

```
summary.lm(model2)
```

```
Call:
aov(formula = root ~ fertilizer, subset = (week == 10))

Residuals:
    Min      1Q  Median      3Q     Max
-0.8167 -0.3667 -0.1333  0.4042  1.4833

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)        9.6167     0.2678  35.915 6.65e-12 ***
fertilizercontrol -1.2833     0.3787  -3.389   0.0069 **

Residual standard error: 0.6559 on 10 degrees of freedom
Multiple R-squared: 0.5346,     Adjusted R-squared: 0.488
F-statistic: 11.49 on 1 and 10 DF,  p-value: 0.006897
```

We can compare this with the output from the `lme`. The effect size in the `lme` is slightly smaller (–1.039 393 compared to –1.2833) but the standard error is appreciably lower (0.203 415 8 compared to 0.3787), so the significance of the result is higher in the `lme` than in the `aov`. You get increased statistical power as a result of going to the trouble of fitting the mixed-effects model. And, crucially, you do not need to make potentially arbitrary judgements about which time period to select for the non-pseudoreplicated analysis. You use all of the data in the model, and you specify its structure appropriately so that the hypotheses are tested with the correct degrees of freedom (10 in this case, not 48).

The reason why the effect sizes are different in the `lm` and `lme` models is that linear models use maximum likelihood estimates of the parameters based on arithmetic means. The linear mixed models, however, use the wonderfully named BLUPs (see p. 685).

19.7 Time series analysis in mixed-effects models

It is common to have repeated measures on subjects in observational studies, where we would expect that the observation on an individual at time $t + 1$ would be quite strongly correlated with the observation on the same individual at time t. This contravenes one of the central assumptions of linear models (p. 503), that the within-group errors are independent. However, we often observe significant serial correlation in data such as these.

The following example comes from Pinheiro and Bates (2000) and forms part of the `nlme` library. The data refer to the numbers of ovaries observed in repeated measures on 11 mares (their oestrus cycles have been scaled such that ovulation occurred at time 0 and at time 1). The issue is how best to model the correlation structure of the data. We know from previous work that the fixed effect can be modelled as a three-parameter sine–cosine function of time x:

$$y = a + b \sin(2\pi x) + d \cos(2\pi x) + \varepsilon_{ij},$$

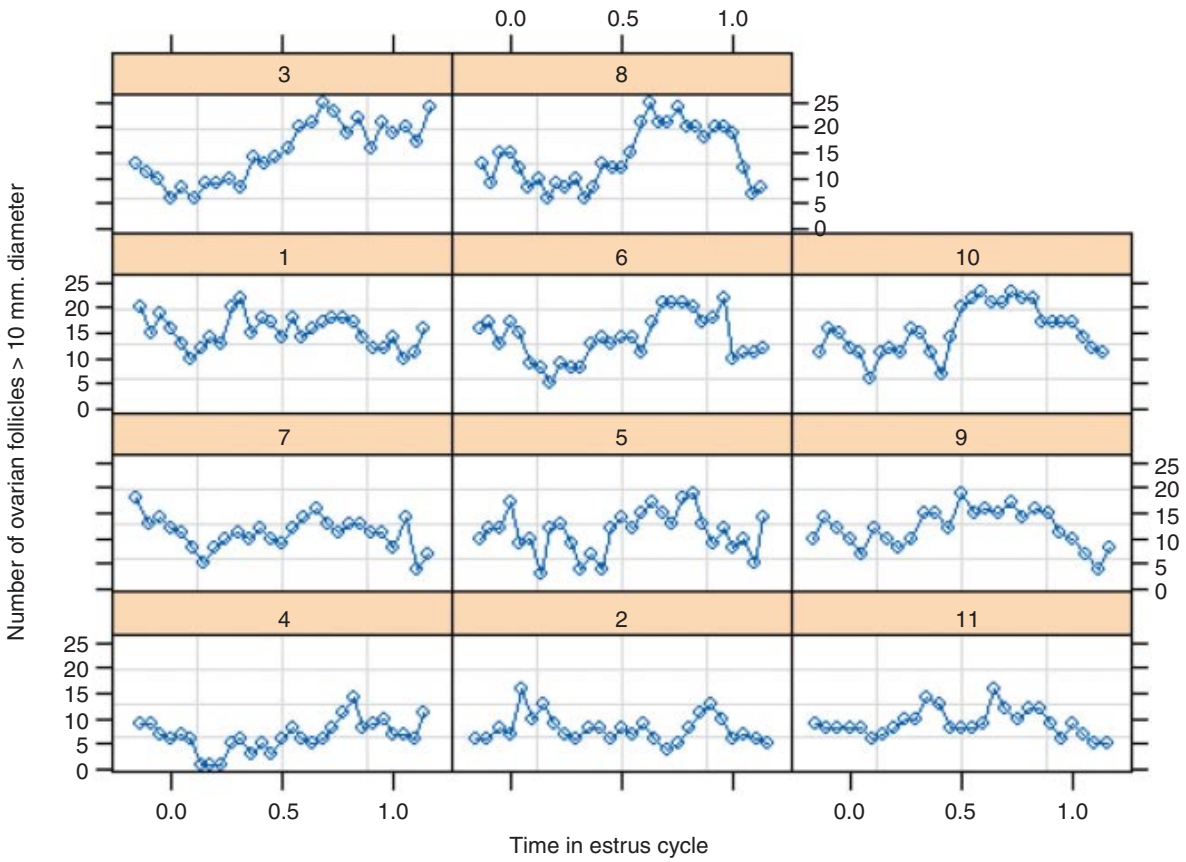
and we want to assess different structures for modelling the within-class correlation.

The dataframe is of class `groupedData` which makes the plotting and error checking much simpler.

```
data(Ovary)
attach(Ovary)
names(Ovary)
```

```
[1]  "Mare"  "Time"  "follicles"
```

```
plot(Ovary)
```



The panel plot has ranked the horses from bottom left to top right on the basis of their mean number of ovules (mare 4 with the lowest number, mare 8 with the highest). Some animals show stronger cyclic behaviour than others.

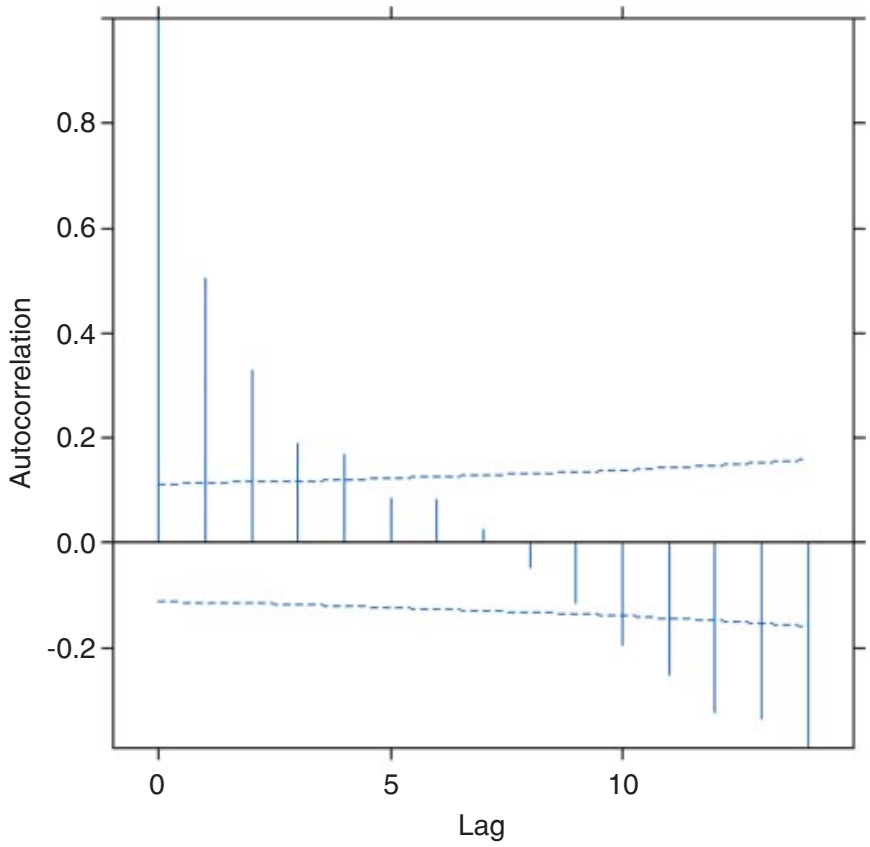
We begin by fitting a mixed-effects model making no allowance for the correlation structure, and investigate the degree of autocorrelation that is exhibited by the residuals (recall that the assumption of the model is that there is no correlation):

```
model <- lme(follicles~sin(2*pi*Time)+cos(2*pi*Time),
                                    data=Ovary,random=~ 1| Mare)
summary(model)
```

```
Linear mixed-effects model fit by REML
 Data: Ovary
      AIC      BIC    logLik
  1669.36 1687.962 -829.6802

Random effects:
 Formula: ~1 | Mare
        (Intercept) Residual
StdDev:    3.041344 3.400466

Fixed effects: follicles ~ sin(2 * pi * Time) + cos(2 * pi * Time)
                       Value Std.Error  DF    t-value p-value
(Intercept)        12.182244 0.9390009 295  12.973623  0.0000
sin(2 * pi * Time) -3.339612 0.2894013 295 -11.539727  0.0000
cos(2 * pi * Time) -0.862422 0.2715987 295  -3.175353  0.0017

Correlation:
                   (Intr) s(*p*T
sin(2 * pi * Time)  0.00
cos(2 * pi * Time) -0.06   0.00

Standardized Within-Group Residuals:
       Min         Q1        Med         Q3        Max
-2.4500138 -0.6721813 -0.1349236  0.5922957  3.5506618

Number of Observations: 308
Number of Groups: 11
```

The function `ACF` allows us to calculate the empirical autocorrelation structure of the residuals from this model:

```
plot(ACF(model),alpha=0.05)
```



You can see that there is highly significant autocorrelation at lags 1 and 2 and marginally significant autocorrelation at lags 3 and 4. We model the autocorrelation structure using one of the standard `corStruct` classes (p. 863). For time series data like this, we typically choose between 'moving average', 'autoregressive' or 'autoregressive moving average' classes. Again, experience with horse biology suggests that a simple moving average model might be appropriate, so we start with this. The class is called `corARMA` and we need to specify the order of the model (the lag of the moving average part). The simplest assumption is that only the first two lags exhibit non-zero correlations (`q=2`):

```
model2 <- update(model,correlation=corARMA(q=2))
anova(model,model2)
```

```
       Model df      AIC      BIC    logLik   Test L.Ratio p-value
model      1  5 1669.360 1687.962 -829.6802
model2     2  7 1574.895 1600.937 -780.4476 1 vs 2 98.4652  <.0001
```

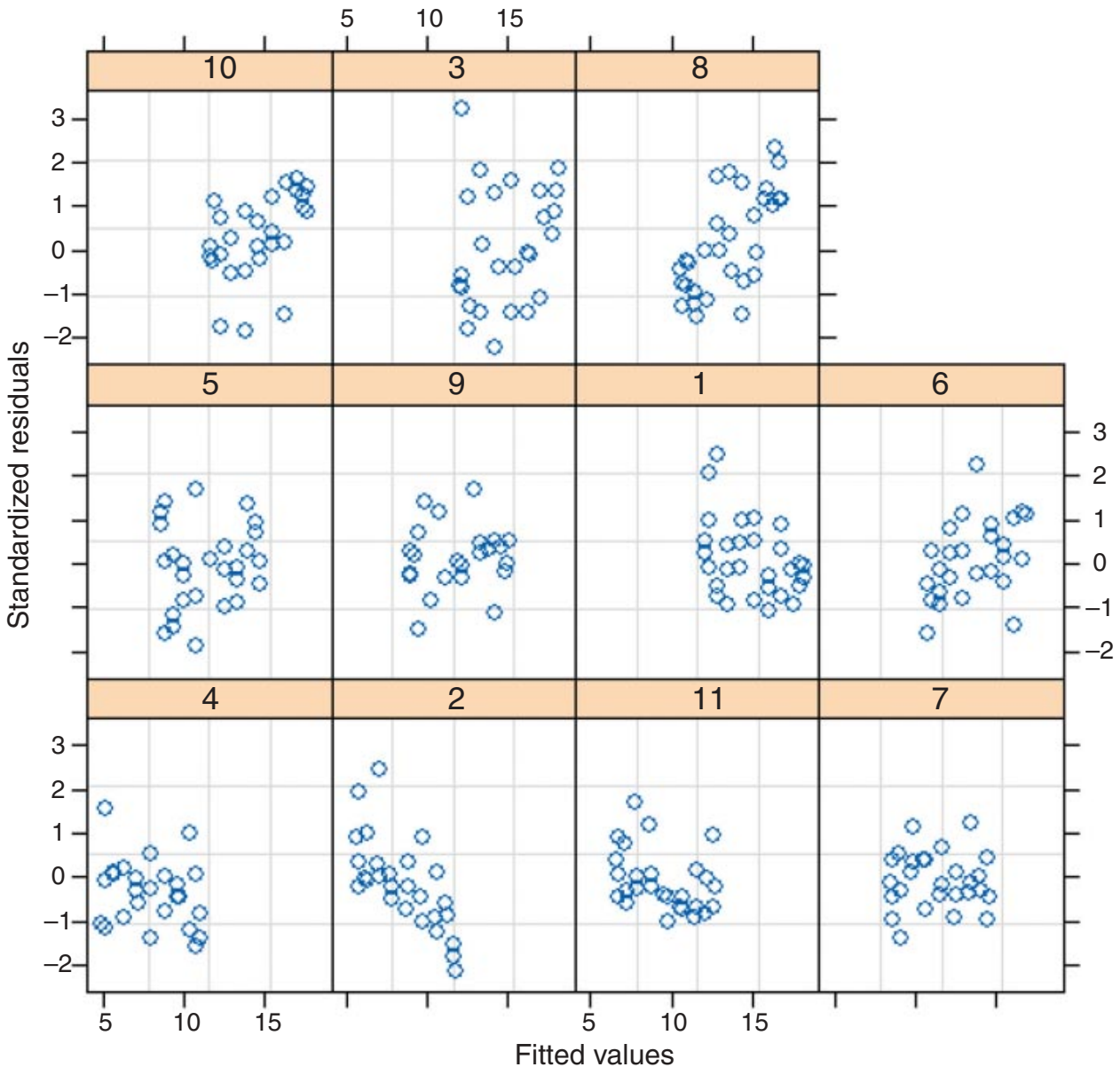
This is a great improvement over the original model, which assumed no correlation in the residuals. But what about a different time series assumption? Let us compare the moving average assumption with a simple first-order autoregressive model `corAR1()`:

```
model3 <- update(model2,correlation=corAR1())
anova(model2,model3)
```

```
       Model df      AIC      BIC    logLik   Test L.Ratio p-value
model2     1  7 1574.895 1600.937 -780.4476
model3     2  6 1562.447 1584.769 -775.2233 1 vs 2 10.4484  0.0012
```

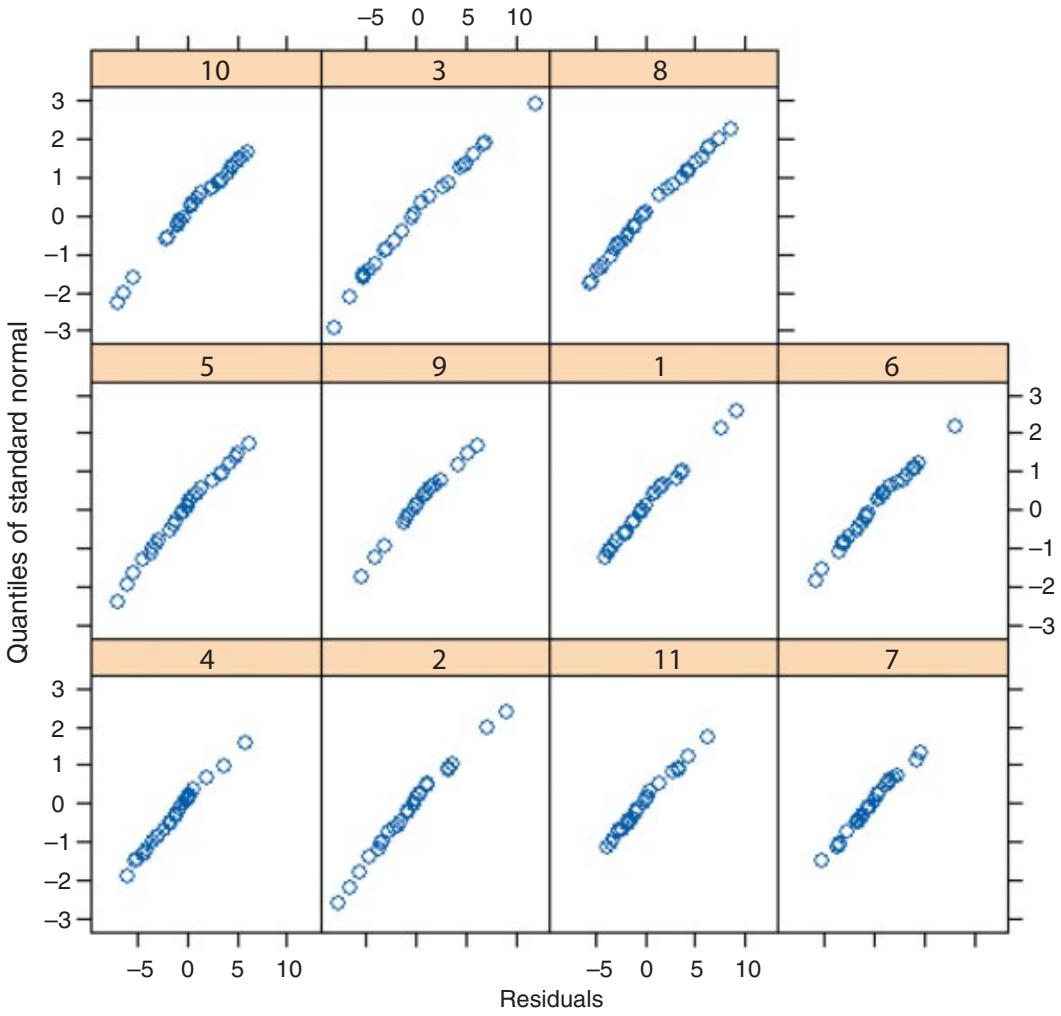
This is a very significant improvement, $p = 0.0012$, so we choose the `corAR1()` because it has the lowest AIC (it also uses fewer degrees of freedom, d.f. = 6). Error checking on `model3` might proceed like this:

```
plot(model3,resid(.,type="p")~fitted(.)|Mare)
```



which shows that residuals are reasonably well behaved. And the normality assumption?

```
qqnorm(model3,~resid(.)|Mare)
```



The errors are close to normally distributed for all of the mares. The model is well behaved, so we accept a first-order autocorrelation structure `corAR1()`.

19.8 Random effects in designed experiments

The rats example, studied by `aov` with an `Error` term on p. 526, can be repeated as a linear mixed-effects model, but only if we recode the factor levels. This example works much better with `lmer` than with `lme`.

```
dd <- read.table("c:\\temp\\rats.txt",h=T)
attach(dd)
names(dd)
```

```
[1]  "Glycogen"  "Treatment" "Rat"   "Liver"
```

```
Treatment <- factor(Treatment)
Liver <- factor(Liver)
Rat <- factor(Rat)
```

There is a single fixed effect (Treatment), and pseudoreplication enters the dataframe because each rat's liver is cut into three pieces and each liver bit is macerated and divided into separate aliquots to produce two readings. First, compute unique factor levels for each rat and each liver bit:

```
rat <- Treatment:Rat
liver <- Treatment:Rat:Liver
```

Then use these as random effects in the lmer model:

```
model <- lmer(Glycogen~Treatment+(1|rat)+(1|liver))
summary(model)
```

```
Linear mixed model fit by REML
Formula: Glycogen ~ Treatment + (1 | rat) + (1 | liver)
   AIC   BIC logLik deviance REMLdev
 231.6 241.1 -109.8    234.3   219.6

Random effects:
 Groups   Name        Variance Std.Dev.
 liver    (Intercept) 14.167   3.7639
 rat      (Intercept) 36.065   6.0054
 Residual             21.167   4.6007

Number of obs: 36, groups: liver, 18; rat, 6

Fixed effects:
            Estimate Std. Error t value
(Intercept)  140.500      4.707  29.850
Treatment2    10.500      6.656   1.577
Treatment3    -5.333      6.656  -0.801
```

You can see that the treatment effect is correctly interpreted as being non-significant (both t values are less than 2 in absolute value). The variance components (p. 527) can be extracted by expressing the variances as percentages:

```
vars <- c(14.167,36.065,21.167)

100*vars/sum(vars)

[1]  19.84201  50.51191  29.64607
```

Thus 50% of the variation is between rats within treatments, 19.8% is between liver bits within rats and 29.6% is between readings within liver bits within rats. If you are interested principally in the fixed effects, then much the best way to proceed is to average away the pseudoreplication and do a one-way ANOVA with 3 d.f. for error (see p. 525).

19.9 Regression in mixed-effects models

The next example involves a regression of plant size against local point measurements of soil nitrogen (N) at five places within each of 24 farms. It is expected that plant size and soil nitrogen will be positively correlated. There is only one measurement of plant size and soil nitrogen at any given point (i.e. there is no temporal pseudoreplication; cf. p. 695):

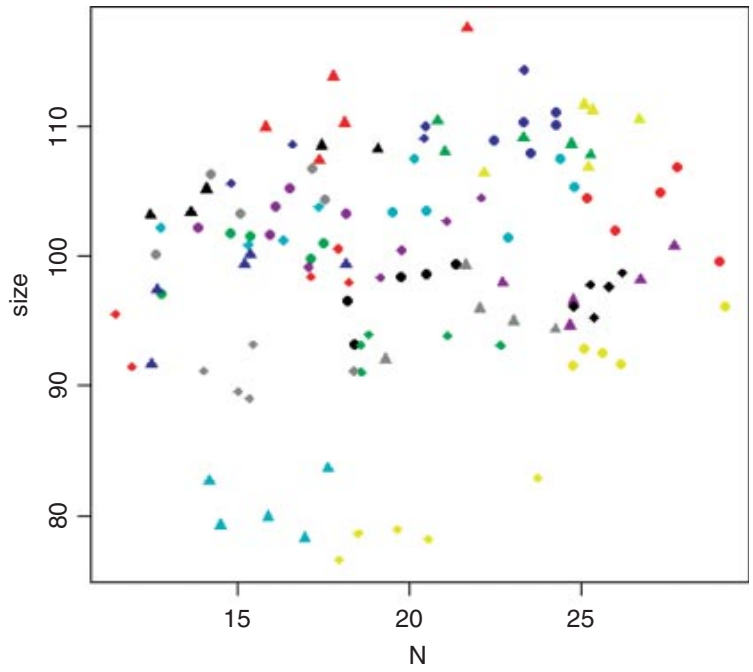
```
yields <- read.table("c:\\temp\\farms.txt",header=T)
attach(yields)
```

```
names(yields)
```

```
[1]  "N"  "size"  "farm"
```

Here are the data in aggregate, with different plotting colours and symbols for each farm:

```
plot(N,size,pch=rep(16:19,each=40),col=farm)
```



The most obvious pattern is that there is substantial variation in mean values of both soil nitrogen and plant size across the farms: the minimum-yielding fields (yellow) have a mean y value of less than 80, while the maximum (red) fields have a mean y value above 110. Note that because there are more farms (24) than colours (8), we need to use different plotting symbols as well as different colours to distinguish the five points from each farm.

The key distinction to understand is between fitting lots of linear regression models (one for each farm) and fitting one mixed-effects model, taking account of the differences between farms in terms of their contribution to the variance in response as measured by a standard deviation in intercept and a standard deviation in slope. We investigate these differences by contrasting the two fitting functions, `lmList` and `lme`. We begin by fitting 24 separate linear models, one for each farm:

```
linear.models <- lmList(size~N|farm,yields)
coef(linear.models)
```

```
   (Intercept)          N
1     67.46260  1.5153805
2    118.52443 -0.5550273
3     91.58055  0.5551292
4     87.92259  0.9212662
5     92.12023  0.5380276
6     97.01996  0.3845431
7     68.52117  0.9339957
8     91.54383  0.8220482
```

```
9     92.04667  0.8842662
10    85.08964  1.4676459
11   114.93449 -0.2689370
12    82.56263  1.0138488
13    78.60940  0.1324811
14    80.97221  0.6551149
15    84.85382  0.9809902
16    87.12280  0.3699154
17    52.31711  1.7555136
18    83.40400  0.8715070
19    88.91675  0.2043755
20    93.08216  0.8567066
21    90.24868  0.7830692
22    78.30970  1.1441291
23    59.88093  0.9536750
24    89.07963  0.1091016
```

You can see very substantial variations in the value of the intercept from 118.52 on farm 2 to 52.32 on farm 17. Slopes are also dramatically different, from negative –0.555 on farm 2 to steep and positive 1.7555 on farm 17. This is a classic problem in regression analysis when (as here) the intercept is a long way from the average value of x (see p. 460); large values of the intercept are almost bound to be correlated with low values of the slope.

Here are the slopes and intercepts from the model specified entirely in terms of random effects: a population of regression slopes predicted within each farm with nitrogen as the continuous explanatory variable, and a population of intercepts for each farm:

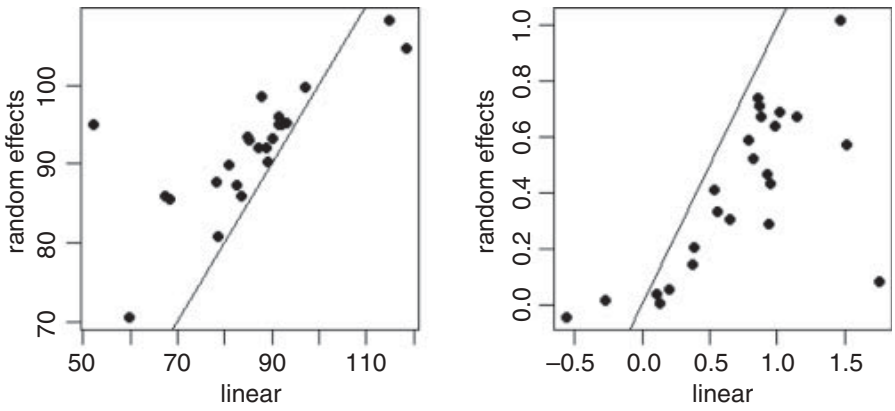
```
random.model <- lme(size~1,random=~N|farm)
coef(random.model)
```

```
   (Intercept)           N
1     85.98139  0.574205332
2    104.67366 -0.045401474
3     95.03442  0.331080929
4     98.62679  0.463579847
5     95.00270  0.407906220
6     99.82294  0.207203700
7     85.57345  0.285520355
8     96.09461  0.520896479
9     95.22186  0.672262931
10    93.14157  1.017995748
11   108.27200  0.015213748
12    87.36387  0.689406424
13    80.83933  0.003617022
14    89.84309  0.306402254
15    93.37050  0.636778731
16    92.10914  0.145772156
17    94.93395  0.084935464
18    85.90160  0.709943272
19    92.00628  0.052485987
```

```
20     95.26296   0.738029408
21     93.35069   0.591151964
22     87.66161   0.673119289
23     70.57827   0.432993929
24     90.29151   0.036747129
```

Differences between the intercepts explain 97.26% of the variance, differences in slope a mere 0.245%, with a residual variance of 2.49% (see the `summary` table). The thing you notice is that the random effects are less extreme (i.e. closer to the mean) than the fixed effects. This is an example of shrinkage (p. 685), and is clearest from a graphical comparison of the coefficients of the linear and mixed models:

```
mm <- coef(random.model)
ll <- coef(linear.models)
windows(7,4)
par(mfrow=c(1,2))
plot(ll[,1],mm[,1],pch=16,xlab="linear",ylab="random effects")
abline(0,1)
plot(ll[,2],mm[,2],pch=16,xlab="linear",ylab="random effects")
abline(0,1)
```



Most of the random-effects intercepts (left) are greater than their linear model equivalents (they are above the 45 degree line) while most of the random-effects slopes (right) are shallower than their linear model equivalents (i.e. below the line). For farm 17 the linear model had an intercept of 52.317 11 while the random-effects model had an intercept of 94.933 95. Likewise, the linear model for farm 17 had a slope of 1.755 513 6 while the random-effects model had a slope of 0.084 935 465.

We can fit a mixed model with both fixed and random effects. Here is a model in which size is modelled as a function of nitrogen and farm as fixed effects, with farm as a random effect. Because we intend to compare models with different fixed effect structures we need to specify `method="ML"` in place of the default REML.

```
farm <- factor(farm)
mixed.model1 <- lme(size~N*farm,random=~1|farm,method="ML")
mixed.model2 <- lme(size~N+farm,random=~1|farm,method="ML")
mixed.model3 <- lme(size~N,random=~1|farm,method="ML")
mixed.model4 <- lme(size~1,random=~1|farm,method="ML")
anova(mixed.model1,mixed.model2,mixed.model3,mixed.model4)
```

```
             Model df      AIC      BIC    logLik   Test   L.Ratio p-value
mixed.model1     1 50 542.9035 682.2781 -221.4518
mixed.model2     2 27 524.2971 599.5594 -235.1486 1 vs 2  27.39359  0.2396
mixed.model3     3  4 614.3769 625.5269 -303.1885 2 vs 3 136.07981  <.0001
mixed.model4     4  3 658.0058 666.3683 -326.0029 3 vs 4  45.62892  <.0001
```

The first model contains a full factorial, with different slopes and intercepts for each of the 25 farms (using up 50 degrees of freedom). The second model has a common slope but different intercepts for the 25 farms (using 27 degrees of freedom); `model2` does not have significantly lower explanatory power than `model1` ($p = 0.2396$). The main effects of farm and of nitrogen application (`model3` and `model4`) are both highly significant ($p < 0.0001$), so we select `model2` because it has the lowest AIC.

Finally, we could do an old-fashioned analysis of covariance, fitting a different two-parameter model to each and every farm without any random effects:

```
model <- lm(size~N*factor(farm))
summary(model)

Call:
lm(formula = size ~ N * factor(farm))

Residuals:
    Min      1Q  Median      3Q     Max
-3.6077 -1.2947  0.0479  1.0732  4.1297

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)       67.46260   14.43749   4.673 1.35e-05 ***
N                  1.51538    0.73395   2.065   0.0426 *
factor(farm)2     51.06183   22.86930   2.233   0.0287 *
factor(farm)3     24.11794   16.54029   1.458   0.1492
factor(farm)4     20.45999   34.59610   0.591   0.5561
factor(farm)5     24.65762   17.29578   1.426   0.1583
factor(farm)6     29.55736   17.74007   1.666   0.1000
factor(farm)7      1.05856   20.53771   0.052   0.9590
factor(farm)8     24.08122   16.23722   1.483   0.1424
factor(farm)9     24.58407   15.45967   1.590   0.1162
factor(farm)10    17.62703   16.68467   1.056   0.2943
factor(farm)11    47.47189   18.24214   2.602   0.0112 *
factor(farm)12    15.10002   15.77085   0.957   0.3415
factor(farm)13    11.14680   17.82896   0.625   0.5338
factor(farm)14    13.50961   19.36739   0.698   0.4877
factor(farm)15    17.39122   20.74850   0.838   0.4047
factor(farm)16    19.66019   18.72739   1.050   0.2973
factor(farm)17   -15.14550   49.01250  -0.309   0.7582
factor(farm)18    15.94140   15.15371   1.052   0.2963
factor(farm)19    21.45414   17.99214   1.192   0.2370
factor(farm)20    25.61956   15.50019   1.653   0.1027
factor(farm)21    22.78608   15.65699   1.455   0.1499
factor(farm)22    10.84710   17.69820   0.613   0.5419
factor(farm)23    -7.58167   16.89435  -0.449   0.6549
```

```
factor(farm)24    21.61703   17.28697   1.250   0.2152
N:factor(farm)2   -2.07041    0.98369  -2.105   0.0388 *
N:factor(farm)3   -0.96025    0.89786  -1.069   0.2884
N:factor(farm)4   -0.59411    1.52204  -0.390   0.6974
N:factor(farm)5   -0.97735    0.84718  -1.154   0.2525
N:factor(farm)6   -1.13084    0.97207  -1.163   0.2485
N:factor(farm)7   -0.58138    0.92164  -0.631   0.5302
N:factor(farm)8   -0.69333    0.87773  -0.790   0.4322
N:factor(farm)9   -0.63111    0.81550  -0.774   0.4415
N:factor(farm)10  -0.04773    0.86512  -0.055   0.9562
N:factor(farm)11  -1.78432    0.87838  -2.031   0.0459 *
N:factor(farm)12  -0.50153    0.84820  -0.591   0.5562
N:factor(farm)13  -1.38290    0.98604  -1.402   0.1651
N:factor(farm)14  -0.86027    0.89294  -0.963   0.3386
N:factor(farm)15  -0.53439    0.94640  -0.565   0.5741
N:factor(farm)16  -1.14547    0.91070  -1.258   0.2125
N:factor(farm)17   0.24013    1.97779   0.121   0.9037
N:factor(farm)18  -0.64387    0.79080  -0.814   0.4182
N:factor(farm)19  -1.31100    0.90886  -1.442   0.1535
N:factor(farm)20  -0.65867    0.78956  -0.834   0.4069
N:factor(farm)21  -0.73231    0.81990  -0.893   0.3747
N:factor(farm)22  -0.37125    0.89597  -0.414   0.6798
N:factor(farm)23  -0.56171    0.85286  -0.659   0.5122
N:factor(farm)24  -1.40628    0.95103  -1.479   0.1436

Residual standard error: 1.978 on 72 degrees of freedom
Multiple R-squared: 0.9678,     Adjusted R-squared: 0.9468
F-statistic: 46.07 on 47 and 72 DF,  p-value: < 2.2e-16
```

There is a marginally significant overall effect of soil nitrogen on plant size (`N` has $p = 0.0426$) and (compared to farm 1) farms 2 and 11 have significantly higher intercepts and shallower slopes. The problem, of course, is that this model, with its 24 slopes and 24 intercepts, is vastly overparameterized. Let us fit a greatly simplified model with a common slope but different intercepts for the different farms:

```
model2 <- lm(size~N+factor(farm))
anova(model,model2)

Analysis of Variance Table

Model 1: size ~ N * factor(farm)
Model 2: size ~ N + factor(farm)
  Res.Df    RSS  Df Sum of Sq      F Pr(>F)
1     72 281.60
2     95 353.81 -23   -72.212 0.8028  0.717
```

This analysis provides no support for any significant differences between slopes. What about differences between farms in their intercepts?

```
model3 <- lm(size~N)
anova(model2,model3)

Analysis of Variance Table
```

```
Model 1: size ~ N + factor(farm)
Model 2: size ~ N
  Res.Df    RSS  Df Sum of Sq      F    Pr(>F)
1     95  353.8
2    118 8454.9 -23   -8101.1 94.574 < 2.2e-16 ***
```

This shows that there are highly significant differences in intercepts between farms. The interpretation of the analysis of covariance is exactly the same as the interpretation of the mixed model in this case where there is balanced structure and equal replication, but `lme` is vastly superior to the linear model when there is unequal replication.

19.10 Generalized linear mixed models

Pseudoreplicated data with non-normal errors lead to a choice of generalized linear mixed-effects models using `lmer` with a specified error family. These were previously handled by the `glmmPQL` function which is part of the `MASS` library (see Venables and Ripley, 2002). That function fitted a generalized linear mixed model with multivariate normal random effects, using penalized quasi-likelihood (hence the 'PQL'). The default method for a generalized linear model fit with `lmer` has been switched from PQL to the more reliable Laplace method. The `lmer` function can deal with the same error structures as a generalized linear model, namely Poisson (for count data), binomial (for binary data or proportion data) or gamma (for continuous data where the variance increase with the square of the mean). The model call is just like a mixed-effects model but with the addition of the name of the error family, like this:

```
lmer(y~fixed+(time | random), family=binomial)
```

For a worked example with binary data, involving patients who were tested for the presence of a bacterial infection on a number of occasions (the number varying somewhat from patient to patient), see pp. 660–665. The response variable is binary: `yes` for infected patients or `no` for patients not scoring as infected, so the family is binomial. There is a single categorical explanatory variable (a fixed effect) called treatment, which has three levels: drug, drug plus supplement, and placebo. The week numbers in which the repeated assessments on each patient were made is also recorded.

19.10.1 Hierarchically structured count data

This is an example of `lmer` with Poisson errors. Beetles were collected in pitfall traps laid out in a grid of two columns and five rows (10 pitfalls per quadrat) in each of two quadrats, randomly located within each of 3 randomly-located blocks within a field. On each of 4 farms there were 5 protocols of hedgerow management allocated at random to each of fields within the farm (1 = uncut control, 2 = grass cut, 3 = grass cut twice, 4 = hedge cut, 5 = grass and hedge cut). Here are the 1200 counts:

```
data<-read.table("c:\\temp\\nested2.txt",header=T)
attach(data)
head(data)
```

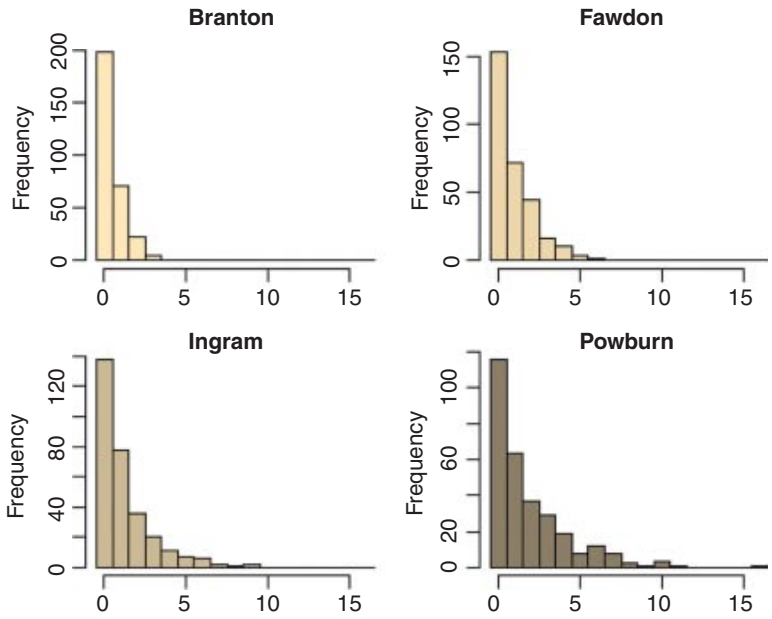
```
count farm field block quadrat
1   1    1     1     1       1
2   0    1     1     1       1
3   0    1     1     1       1
4   1    1     1     1       1
```

```
5    1     1      1      1        1
6    0     1      1      1        1
```

```
farm<-factor(farm)
field <- factor(field)
block <- factor(block)
quadrat <- factor(quadrat)
library(lme4)
```

This is what the data look like, classified by farms:

```
par(mfrow=c(2,2))
hist(count[farm==1],breaks=-0.5:16.5,
            main="Branton",col="wheat1",xlab="")
hist(count[farm==2],breaks=-0.5:16.5,
            main="Fawdon",col="wheat2",xlab="")
hist(count[farm==3],breaks=-0.5:16.5,
            main="Ingram",col="wheat3",xlab="")
hist(count[farm==4],breaks=-0.5:16.5,
            main="Powburn",col="wheat4",xlab="")
```



Most of the pitfall traps contained no beetles at all sites, and the maximum number caught was 16 on one occasion at Powburn. There are large differences between the farms in mean beetle counts, but despite this, the five hedgerow management treatments showed substantial differences:

```
tapply(count,list(farm,field),mean)
```

```
          1         2         3         4         5
1 0.4000000 0.4666667 0.4500000 0.5833333 0.5833333
2 0.6833333 0.7666667 0.7833333 1.2000000 1.1333333
3 0.8500000 0.9166667 1.6666667 1.2500000 1.3833333
4 1.5000000 2.2166667 1.8000000 1.4500000 2.2000000
```

We need to establish whether the treatment differences (as shown here between the column means) are significant. Because of the massive pseudoreplication, we need to analyse the counts very carefully. We shall use a generalized mixed effects model with Poisson errors, treating field as a fixed effect (the `field` codes refer to the five hedgerow management treatments) with the other factors as nested random effects (quadrats within blocks, within fields, within farms):

```
model <- lmer(count~field+(1|farm/field/block/quadrat),family=poisson)
summary(model)
```

```
Generalized linear mixed model fit by the Laplace approximation
Formula: count ~ field + (1 | farm/field/block/quadrat)
  AIC  BIC logLik deviance
 2216 2262  -1099     2198

Random effects:
 Groups                        Name        Variance Std.Dev.
 quadrat:(block:(field:farm)) (Intercept) 0.133785 0.36577
 block:(field:farm)           (Intercept) 0.017054 0.13059
 field:farm                   (Intercept) 0.000000 0.00000
 farm                         (Intercept) 0.216319 0.46510

Number of obs: 1200, groups: quadrat:(block:(field:farm)), 120;
block:(field:farm), 60; field:farm, 20; farm, 4

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  -0.3322     0.2586  -1.285  0.19889
field2        0.2202     0.1561   1.411  0.15830
field3        0.2851     0.1552   1.836  0.06632 .
field4        0.3205     0.1546   2.074  0.03811 *
field5        0.4402     0.1533   2.872  0.00407 **
```

As you can see, management treatments 4 and 5 produced significantly higher beetle counts than the controls. Among the random effects you will see that `field:farm` (the fixed effect) registers as zero. Much the biggest cause of variation in beetle numbers was differences between the four farms (variance = 0.216) , then differences between the quadrats within a block (0.134), with only very modest differences from block to block within each field (0.017).

If, instead of fitting the management treatment as a fixed effect, we were to fit is as a random effect, then the model changes as follows:

```
model2 <- lmer(count~1+(1|farm/field/block/quadrat),family=poisson)
summary(model2)
```

```
Generalized linear mixed model fit by the Laplace approximation
Formula: count ~ 1 + (1 | farm/field/block/quadrat)
  AIC  BIC logLik deviance
 2216 2242  -1103     2206

Random effects:
 Groups                        Name        Variance Std.Dev.
 quadrat:(block:(field:farm)) (Intercept) 0.133591 0.365501
 block:(field:farm)           (Intercept) 0.035101 0.187354
```

```
 field:farm                    (Intercept) 0.001888 0.043451
 farm                          (Intercept) 0.213685 0.462260

Number of obs: 1200, groups: quadrat:(block:(field:farm)), 120;
block:(field:farm), 60; field:farm, 20; farm, 4

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) -0.07779    0.23703  -0.328    0.743
```

The variance component attributable to differences between blocks has almost doubled (this is the spatial scale immediately below fields, which was the scale at which the fixed effects treatments were applied), but the farm-scale variation (larger scale) and quadrat-scale variation (smaller scale) are almost unaffected. So the difference between fields which looks so small as a random effect (0.001 888) is clearly significant when the term is fitted as a fixed effect (as was appropriate in this example).

Here is the much simpler analysis with the pseudoreplication averaged away. Now, there are only 20 numbers in the dataframe (5 numbers (the five treatment mean counts) from each of 4 farms), and we use these to create a new response variable:

```
y <- as.vector(tapply(count,list(farm,field),mean))
y
```

```
 [1] 0.4000000 0.6833333 0.8500000 1.5000000 0.4666667 0.7666667 0.9166667
 [8] 2.2166667 0.4500000 0.7833333 1.6666667 1.8000000 0.5833333 1.2000000
[15] 1.2500000 1.4500000 0.5833333 1.1333333 1.3833333 2.2000000
```

We also need new shorter vectors of categorical explanatory variables for the farm (which now serves as a four-level block in this two-way, non-replicated analysis) and the five-level hedgerow management treatment. The 'generate factor levels' function, `gl`, is useful here. For farm blocks (`fblock`) we read the instruction to `gl` like this: 'generate levels up to 4, each with a repeat of 1, to a total length of 20'. For `hedge` it says 'generate levels up to 5, each with a repeat of 4'.

```
fblock <- gl(4,1,20)
hedge <- gl(5,4)
```

We cannot fit an exactly analogous model because we no longer have count data (the average beetle numbers are all real numbers) so we cannot use `glm` with Poisson errors. We can get close enough, however, by using a linear model with the logarithms of the mean counts as the response variable like this:

```
model3 <- lm(log(y)~fblock+hedge)
summary(model3)
```

```
Call:
lm(formula = log(y) ~ fblock + hedge)
Residuals:
     Min       1Q   Median       3Q      Max
-0.29679 -0.08192 -0.01837  0.05106  0.31607

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  -0.9555     0.1179  -8.102 3.30e-06 ***
fblock2       0.5943     0.1179   5.039  0.00029 ***
fblock3       0.8728     0.1179   7.400 8.27e-06 ***
```

```
fblock4       1.3008     0.1179  11.030 1.23e-07 ***
hedge2        0.1838     0.1319   1.394  0.18859
hedge3        0.2775     0.1319   2.105  0.05708 .
hedge4        0.3230     0.1319   2.450  0.03060 *
hedge5        0.4383     0.1319   3.324  0.00606 **

Residual standard error: 0.1865 on 12 degrees of freedom
Multiple R-squared: 0.9214,     Adjusted R-squared: 0.8756
F-statistic:  20.1 on 7 and 12 DF,  p-value: 9.875e-06
```

The *p* values are slightly different, but the interpretation is exactly the same. After model simplification, it turns out that there is one highly significant contrast, between the last two hedge management treatments (4 and 5) and the rest ($p = 0.0102$). It is hedge cutting, not grass cutting, that makes the difference in beetle counts in this case.

An alternative method of producing the reduced dataframe (i.e. eliminating the pseudoreplication) is to use `aggregate` like this:

```
d2<-aggregate(data,list(farm,field),mean)
model<-lm(log(count)~factor(farm)+factor(field),data=d2)
summary(model)
```

This produces the same output with less than half the R code.