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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. 473). 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

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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.

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 two model formulae;
- 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 don't 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. 645). 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.'

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 each treatment 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 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.

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 (see the Index for worked examples of lmer in this book; all of the analyses in this chapter using lme are repeated using lmer on the book's website). Here, I provide a simple comparison of the basic syntax of the two functions.

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. The random effects show the identities of the random variables and their relative locations in the hierarchy. The random effects 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 $\sim$. 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 (this is parameter 1) 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)
```

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))
```

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, then the fixed effects and the BLUP are similar (i.e. when most of the variation is between classes and there is little variation within classes). 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 (p. 547).

A Designed Experiment 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. 470).

```
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 (which is parameter 1) as random=~1. Finally, we define the spatial structure of the random effects after the 'given' symbol | as: block/irrigation/density. 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: 0.0006601056

Formula: ~ 1 | irrigation %in% block
     (Intercept)
StdDev: 1.982461

Formula: ~ 1 | density %in% irrigation %in% block
     (Intercept)  Residual
StdDev: 6.975554  9.292805

Fixed effects: yield ~ irrigation * density * fertilizer
                        Value    Std.Error   DF     t-value
(Intercept)             80.50     5.893741   36   13.658558
irrigationirrigated     31.75     8.335008    3    3.809234
densitylow               5.50     8.216282   12    0.669403
```

```
densitymedium                                14.75   8.216282  12   1.795216
fertilizerNP                                  5.50   6.571005  36   0.837010
fertilizerP                                   4.50   6.571005  36   0.684827
irrigationirrigated:densitylow              -39.00  11.619577  12  -3.356404
irrigationirrigated:densitymedium           -22.25  11.619577  12  -1.914872
irrigationirrigated:fertilizerNP             13.00   9.292805  36   1.398932
irrigationirrigated:fertilizerP               5.50   9.292805  36   0.591856
densitylow:fertilizerNP                       3.25   9.292805  36   0.349733
densitymedium:fertilizerNP                   -6.75   9.292805  36  -0.726368
densitylow:fertilizerP                       -5.25   9.292805  36  -0.564953
densitymedium:fertilizerP                    -5.50   9.292805  36  -0.591856
irrigationirrigated:densitylow:fertilizerNP   7.75  13.142011  36   0.589712
irrigationirrigated:densitymedium:fertilizerNP 3.75 13.142011  36   0.285344
irrigationirrigated:densitylow:fertilizerP   20.00  13.142011  36   1.521837
irrigationirrigated:densitymedium:fertilizerP 4.00  13.142011  36   0.304367
                                           p-value
(Intercept)                                 0.0000
irrigationirrigated                         0.0318
densitylow                                  0.5159
densitymedium                               0.0978
fertilizerNP                                0.4081
fertilizerP                                 0.4978
irrigationirrigated:densitylow              0.0057
irrigationirrigated:densitymedium           0.0796
irrigationirrigated:fertilizerNP            0.1704
irrigationirrigated:fertilizerP             0.5576
densitylow:fertilizerNP                     0.7286
densitymedium:fertilizerNP                  0.4723
densitylow:fertilizerP                      0.5756
densitymedium:fertilizerP                   0.5576
irrigationirrigated:densitylow:fertilizerNP 0.5591
irrigationirrigated:densitymedium:fertilizerNP 0.7770
irrigationirrigated:densitylow:fertilizerP  0.1368
irrigationirrigated:densitymedium:fertilizerP 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: 0.000563668
```

```
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: 0.0005569251

Formula: ~ 1 | irrigation %in% block
     (Intercept)
StdDev:  1.982615

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   8.800160  12  -3.380620   0.0055
irrigationirrigated:densitymedium -19.66667   8.800160  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
```

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") 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–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–density interaction ($p = 0.0029$ compared to $p = 0.016\,33$). 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:  0.0005261129

Formula: ~ 1 | irrigation %in% block
     (Intercept)
StdDev:  1.716889

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
```

Note that the degrees of freedom are not pseudoreplicated: d.f. = 12 for testing the irrigation by density interaction and d.f. = 44 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 compare models with different fixed-effect structures using REML.

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

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

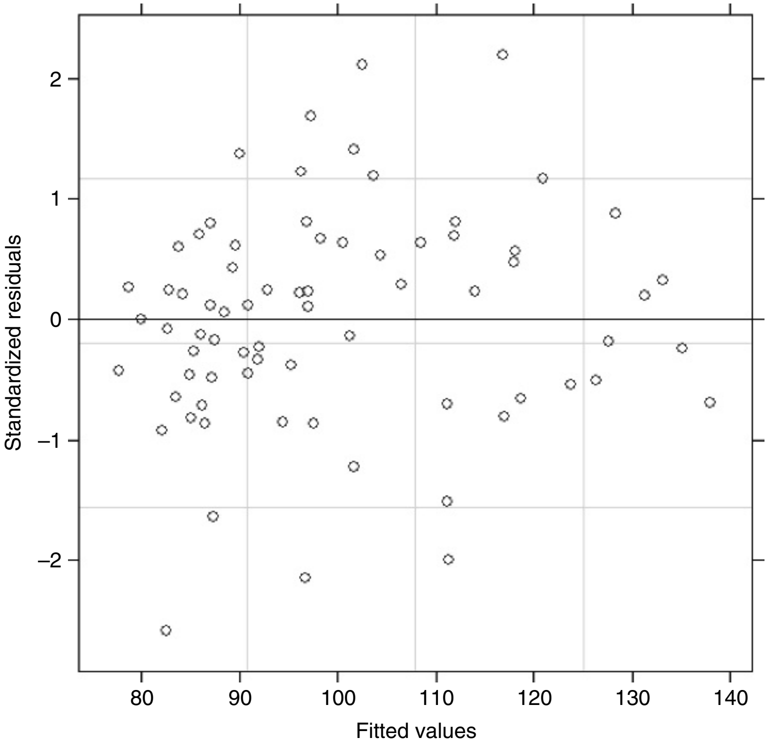
The response variable is a reasonably linear function of the fitted values:

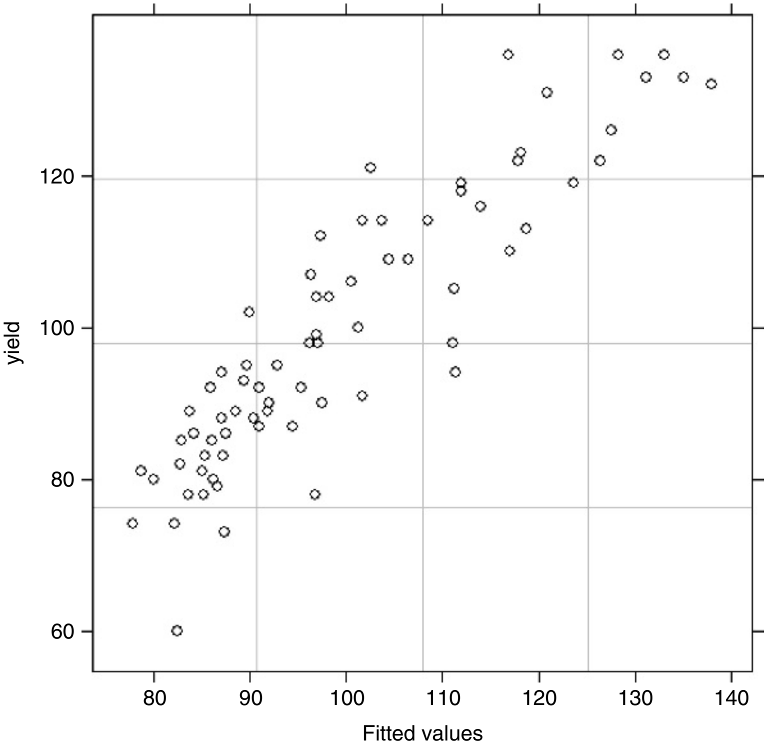
```
plot(model.lme.3,yield~fitted(.))
```

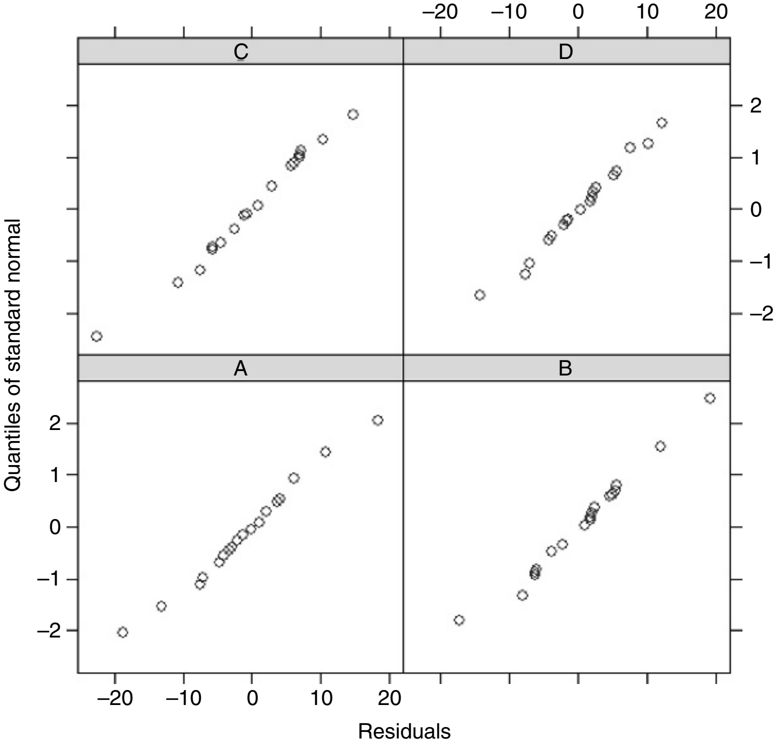
and the errors are reasonably close to normally distributed in all four blocks:

```
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. 470). Without balance, however, you will need to use lme and to use model simplification to estimate the *p* values of the significant interaction terms.


Standardized residuals
2
1
0
−1
−2
80
90
100
110
120
130
140
Fitted values


yield
120
100
80
60
80
90
100
110
120
130
140
Fitted values



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. Consider an epidemiological study of childhood diseases in which blood samples were taken for individual children, households, streets, districts, towns, regions, and countries. All these categorical variables are random effects. The spatial scale increases with each step in the hierarchy. The interest lies in discovering where most of the variation originates: is it between children within households or between districts within the same town? When it comes to testing hypotheses at larger spatial scales (such as town or regions), such data sets contain huge amounts of pseudoreplication.

The following example has a slightly simpler spatial structure than this: infection is measured for two replicate males and females within each of three families within four streets within three districts within five towns (720 measurements in all). We want to carry out a variance components analysis. Here are the data:

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

```
[1] "subject"  "town"       "district"   "street"   "family"
[6] "gender"   "replicate"
```

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

```
model1<-lme(subject~1,random=~1|town/district/street/family/gender)
summary(model1)
```

```
Linear mixed-effects model fit by REML
Data: NULL
     AIC       BIC     logLik
3351.294  3383.339  -1668.647

Random effects:
Formula: ~1 | town
     (Intercept)
StdDev: 1.150604

Formula: ~1 | district %in% town
     (Intercept)
StdDev: 1.131932

Formula: ~1 | street %in% district %in% town
     (Intercept)
StdDev: 1.489864

Formula: ~1 | family %in% street %in% district %in% town
     (Intercept)
StdDev: 1.923191

Formula: ~1 | gender %in% family %in% street %in% district %in% town
     (Intercept)  Residual
StdDev: 3.917264  0.9245321

Fixed effects: subject ~ 1
                Value  Std.Error   DF   t-value  p-value
(Intercept)  8.010941  0.6719753  360  11.92148        0

Standardized Within-Group Residuals:
        Min           Q1          Med          Q3         Max
-2.64600654  -0.47626815  -0.06009422  0.47531635  2.35647504

Number of Observations: 720
Number of Groups:
                                                  town
                                                     5
                                    district %in% town
                                                    15
                          street %in% district %in% town
                                                    60
                family %in% street %in% district %in% town
                                                   180
gender %in% family %in% street %in% district %in% town
                                                   360
```

Notice that the model was fitted by REML rather than by the more familiar maximum likelihood. REML methods differ because they allow for the degrees of freedom used up in estimating the fixed effects. Thus, the variance components are estimated without being affected by the fixed effects (they are invariant to the values of the fixed effects). Also, REML estimators are less sensitive to outliers than are ML estimators.

To calculate the variance components we need to extract the standard deviations of the random effects from the model summary, square them to get the variances, then express each as a percentage of the total:

```
sds<-c(1.150604,1.131932,1.489864,1.923191,3.917264,0.9245321)
vars<-sds^2
100*vars/sum(vars)
```

```
[1]  5.354840  5.182453  8.978173  14.960274  62.066948  3.457313
```

This indicates that the gender effect (62%) is much the most important component of overall variance. Next most important is variation from family to family (15%).

For comparison, here is the layout of the output for the same analysis using lmer:

```
library(lme4)
model1<-lmer(subject~1+(1|town/district/street/family/gender))
summary(model1)
```

```
Linear mixed-effects model fit by REML
Formula: subject ~ 1 + (1 | town/district/street/family/gender)
 AIC    BIC   logLik  MLdeviance  REMLdeviance
3349   3377    -1669        3338          3337

Random effects:
  Groups                                    Name         Variance  Std.Dev.
  gender:(family:(street:(district:town)))  (Intercept)   15.3387   3.91647
  family:(street:(district:town))           (Intercept)    3.7008   1.92375
  street:(district:town)                    (Intercept)    2.2283   1.49274
  district:town                             (Intercept)    1.2796   1.13121
  town                                      (Intercept)    1.3238   1.15056
  Residual                                                 0.8548   0.92456

number of obs: 720, groups: gender:(family:(street:(district:town))),
360; family:(street:(district:town)), 180; street:(district:town), 60;
district:town, 15; town, 5

Fixed effects:
             Estimate  Std. Error  t value
(Intercept)     8.011       0.672    11.92
```

You will see that the variance components are given in the penultimate column. Fixed effects in this model are discussed on p. 656.

Model Simplification in Hierarchical Sampling

We need to know whether all of the random effects are required in the model. The key point to grasp here is that you will need to recode the factor levels if you want to leave out a random effect from a larger spatial scale. Suppose we want to test the effect of leaving out the identity of the towns. Because the districts were originally coded with the same names within each town,

```
levels(district)
```

```
[1]  "d1"  "d2"  "d3"
```

we shall need to create 15 new, unique district names. Much the simplest way to do this is to use paste to combine the town names and the district names:

```
newdistrict<-factor(paste(town,district,sep=""))
levels(newdistrict)
```

```
 [1] "Ad1"  "Ad2"  "Ad3"  "Bd1"  "Bd2"  "Bd3"  "Cd1"  "Cd2"  "Cd3"  "Dd1"
[11] "Dd2"  "Dd3"  "Ed1"  "Ed2"  "Ed3"
```

In model2 we leave out the random effect for towns and include the new factor for districts:

```
model2<-lme(subject~1,random=~1|newdistrict/street/family/gender)
anova(model1,model2)
```

```
       Model df      AIC      BIC    logLik   Test  L.Ratio p-value
model1     1  7 3351.294 3383.339 -1668.647
model2     2  6 3350.524 3377.991 -1669.262 1 vs 2 1.229803  0.2674
```

Evidently there is no significant effect attributable to differences between towns ($p = 0.2674$).

The next question concerns differences between the districts. Because the streets within districts were all coded in the same way in the original dataframe, we need to create 60 unique codes for the different streets:

```
newstreet<-factor(paste(newdistrict,street,sep=""))
levels(newstreet)
```

```
 [1] "Ad1s1" "Ad1s2" "Ad1s3" "Ad1s4" "Ad2s1" "Ad2s2" "Ad2s3" "Ad2s4" "Ad3s1"
[10] "Ad3s2" "Ad3s3" "Ad3s4" "Bd1s1" "Bd1s2" "Bd1s3" "Bd1s4" "Bd2s1" "Bd2s2"
[19] "Bd2s3" "Bd2s4" "Bd3s1" "Bd3s2" "Bd3s3" "Bd3s4" "Cd1s1" "Cd1s2" "Cd1s3"
[28] "Cd1s4" "Cd2s1" "Cd2s2" "Cd2s3" "Cd2s4" "Cd3s1" "Cd3s2" "Cd3s3" "Cd3s4"
[37] "Dd1s1" "Dd1s2" "Dd1s3" "Dd1s4" "Dd2s1" "Dd2s2" "Dd2s3" "Dd2s4" "Dd3s1"
[46] "Dd3s2" "Dd3s3" "Dd3s4" "Ed1s1" "Ed1s2" "Ed1s3" "Ed1s4" "Ed2s1" "Ed2s2"
[55] "Ed2s3" "Ed2s4" "Ed3s1" "Ed3s2" "Ed3s3" "Ed3s4"
```

Now fit the new model leaving out both towns and districts,

```
model3<-lme(subject~1,random=~1|newstreet/family/gender)
```

and compare this with model2 from which towns had been removed:

```
anova(model2,model3)
```

```
       Model df      AIC      BIC    logLik   Test  L.Ratio p-value
model2     1  6 3350.524 3377.991 -1669.262
model3     2  5 3354.084 3376.973 -1672.042 1 vs 2 5.559587  0.0184
```

This simplification was not justified ($p = 0.0184$) so we conclude that there is significant variation from district to district. Model-checking plots are illustrated on p. 657.

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. 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 (created by read.table) into a groupedData object (p. 668). 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:

```
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, ranked from bottom left to top right 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 two panels, one for the fertilized plants and one for the controls. This is easy:

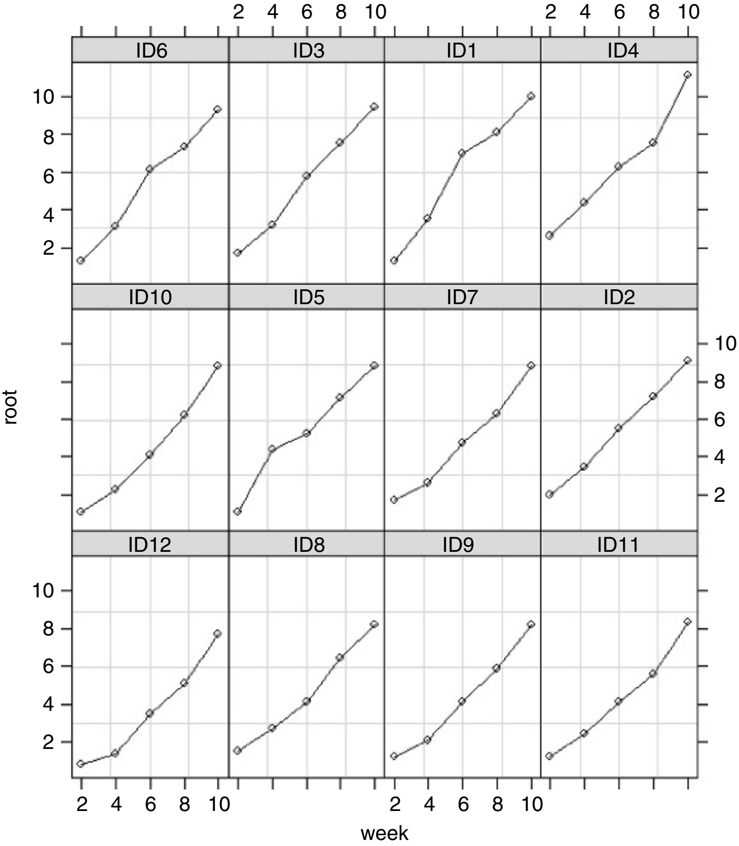
```
plot(results,outer=T)
```

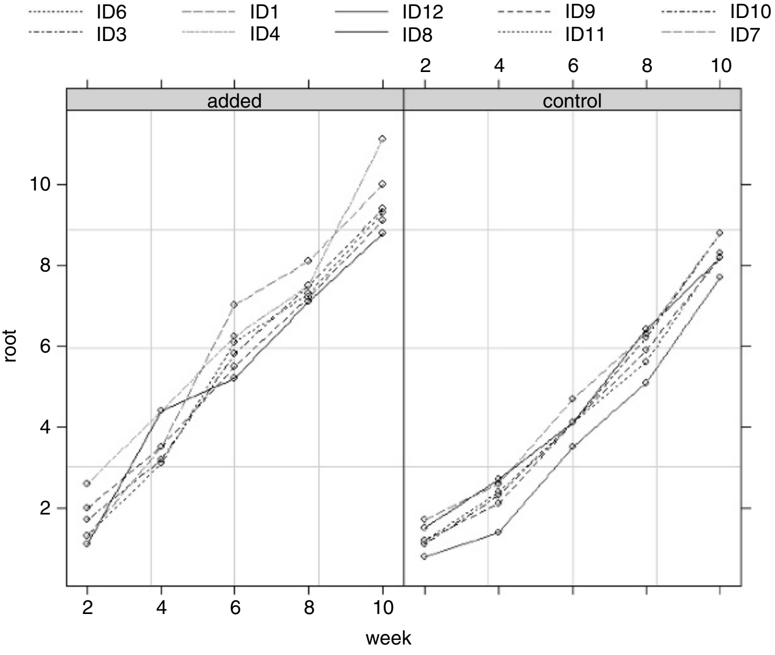
You can see that by week 10 there is virtually no overlap between the two treatment groups. The largest control plant has about the same root length as the smallest fertilized plant (*c*.9 cm).

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
```


ID6
ID3
ID1
ID4
ID10
ID5
ID7
ID2
ID12
ID8
ID9
ID11
root
week
2
4
6
8
10


ID6
ID3
ID1
ID4
ID12
ID8
ID9
ID11
ID10
ID7
added
control
root
week
2
4
6
8
10

```
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.?

Here is a simple one-way ANOVA for the non-pseudoreplicated data from week 10:

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

```
              Df   Sum Sq   Mean Sq   F value     Pr(>F)
fertilizer     1   4.9408    4.9408    11.486   0.006897   **
Residuals     10   4.3017    0.4302
```

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

```
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   **
```

We can compare this with the output from the lme. The effect size in the lme is slightly smaller (−1.039393 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.

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 mixed-effects models (p. 627), 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

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

and we want to assess different structures for modelling the within-class correlation structure. The dataframe is of class groupedData which makes the plotting and error checking much simpler.

```
library(nlme)
library(lattice)
data(Ovary)
attach(Ovary)
names(Ovary)
```

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

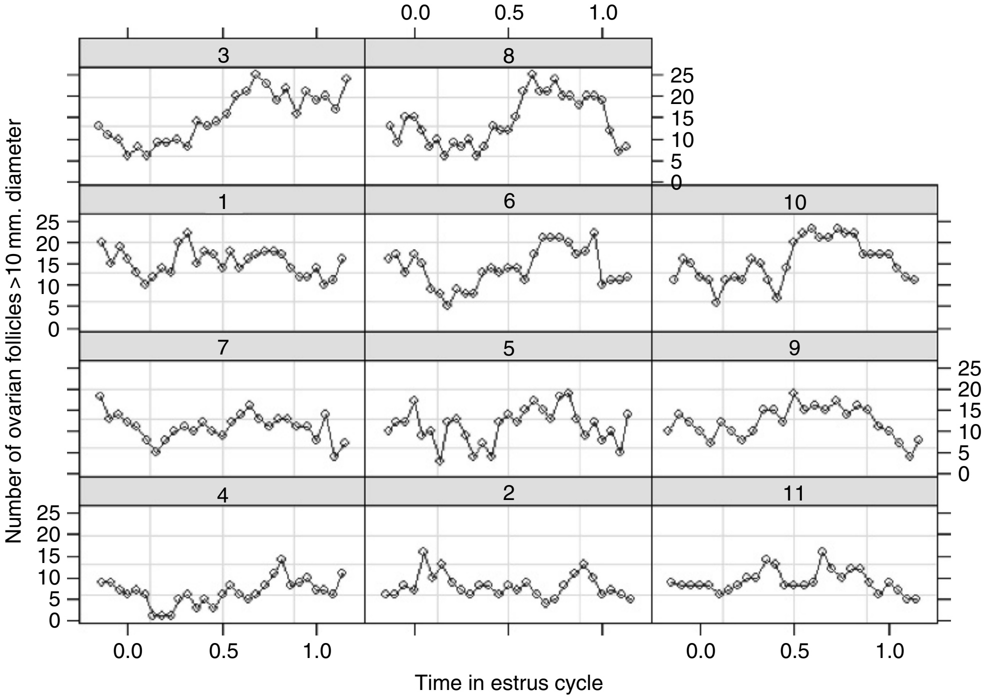
```
plot(Ovary)
```

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
1669.360  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)
```

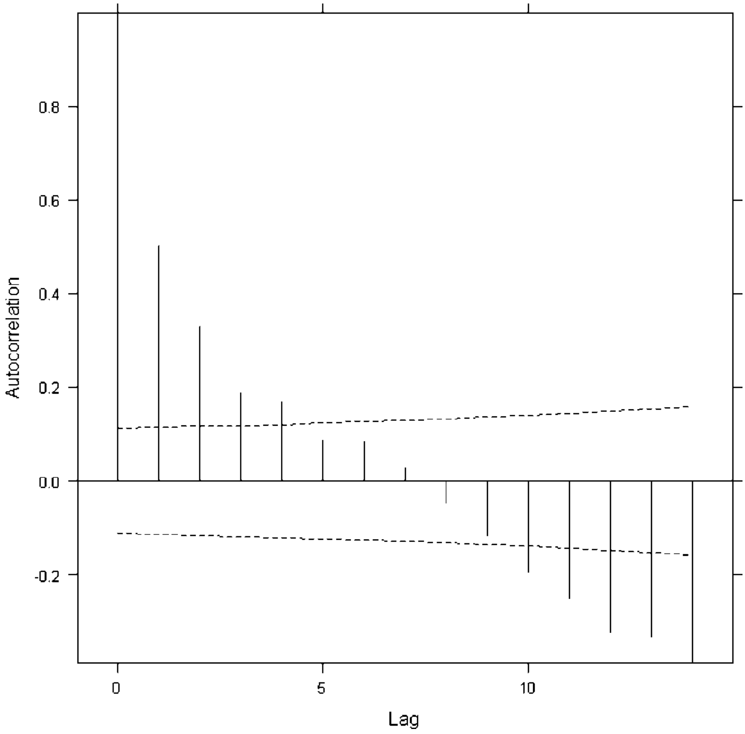


```
                        Value   Std.Error   DF      t-value   p-value
(Intercept)         12.182244   0.9390010  295    12.973623    0.0000
sin(2 * pi * Time)  -3.339612   0.2894013  295   -11.539727    0.0000
cos(2 * pi * Time)  -0.862422   0.2715987  295    -3.175353    0.0017

 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. 701). 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  1573.453  1595.775  -780.7264  1 vs 2  0.5577031   0.4552
```

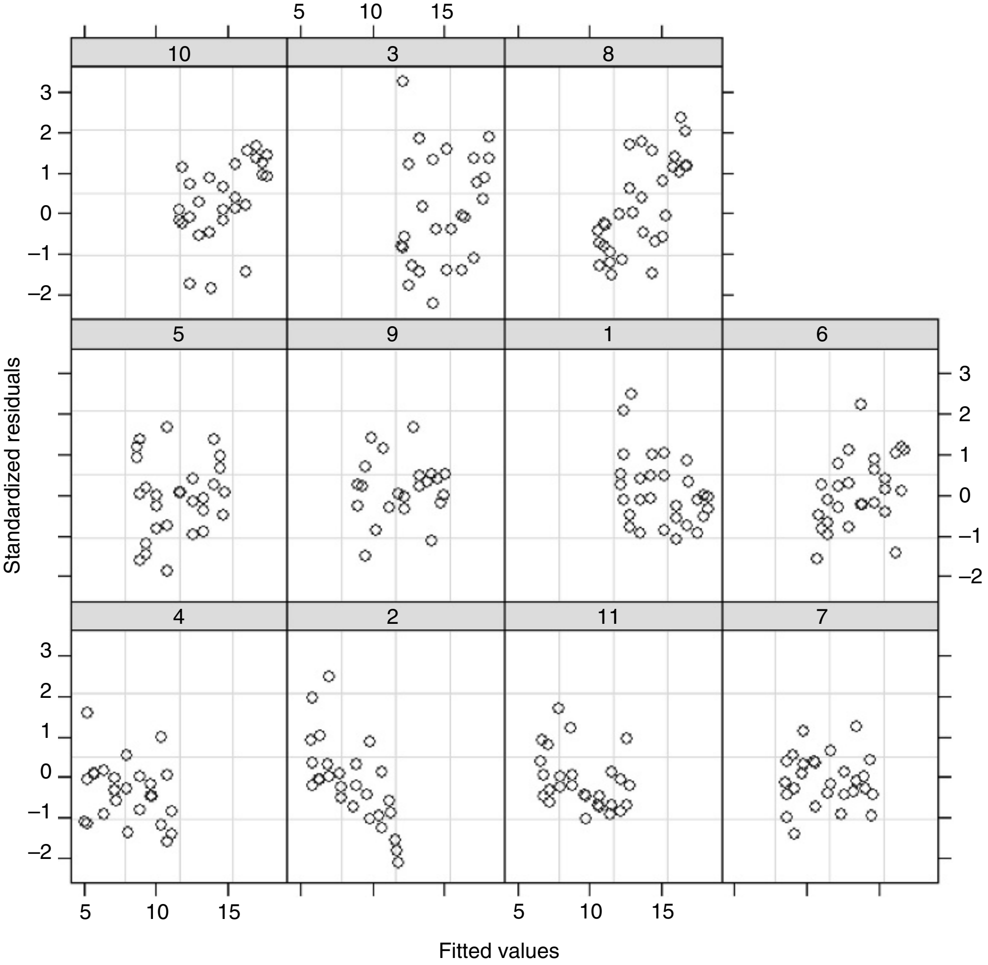
There is nothing to chose between the models on the basis of ANOVA, $p = 0.455$, so we choose the corAR1() because it has the lowest AIC (it also uses fewer degrees of freedom, d.f. $= 6$). Error checking on model3 might proceed like this:

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

The residuals appear to be reasonably well behaved. And the normality assumption?

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

The model is well behaved, so we accept a first-order autocorrelation structure corAR1().



Random Effects in Designed Experiments

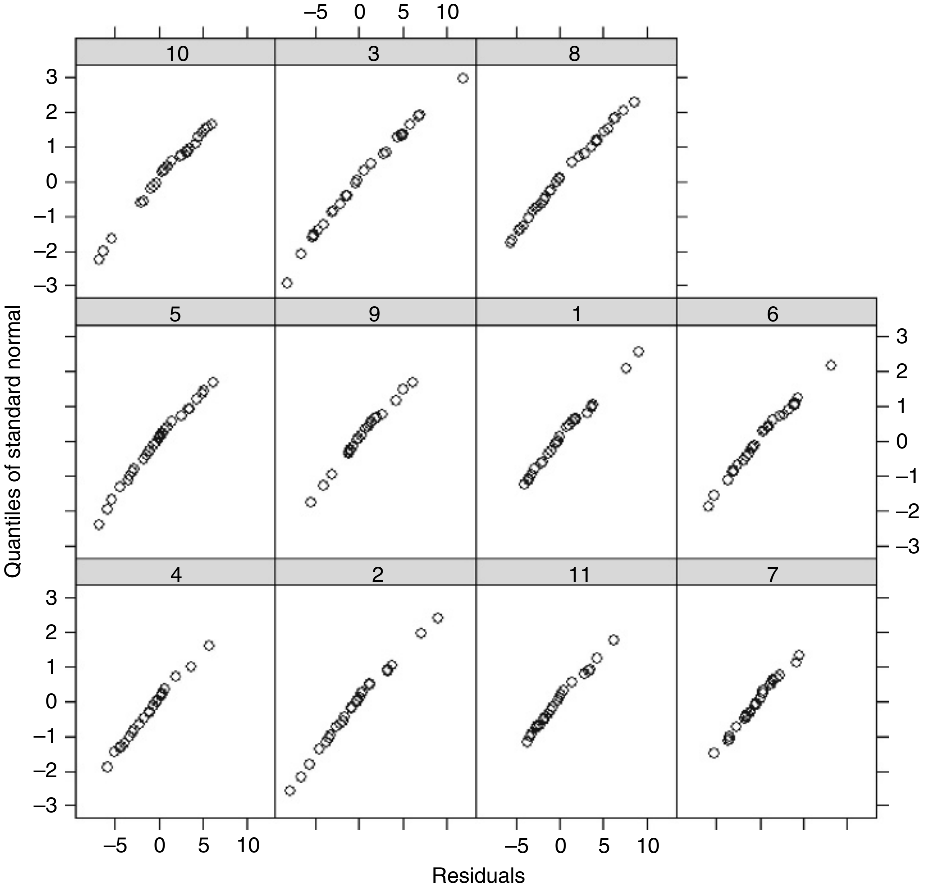
The rats example, studied by aov with an Error term on p. 476, can be repeated as a linear mixed-effects model. This example works much better with lmer than with lme.

```
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 separate liver bit produces two readings.



The rats are numbered 1 and 2 within each treatment, so we need Treatment as the largest scale of the random effects.

```
model<-lmer(Glycogen~Treatment+(1|Treatment/Rat/Liver))
summary(model)
```

```
Linear mixed-effects model fit by REML
Formula: Glycogen ~ Treatment + (1 | Treatment/Rat/Liver)

  AIC    BIC  logLik  MLdeviance  REMLdeviance
231.6  241.1  -109.8       234.9         219.6

Random effects:
Groups                 Name         Variance  Std.Dev.
Liver:(Rat:Treatment)  (Intercept)   14.1617    3.7632
Rat:Treatment          (Intercept)   36.0843    6.0070
Treatment              (Intercept)    4.7039    2.1689
Residual                             21.1678    4.6008

number of obs: 36, groups: Liver:(Rat:Treatment), 18; Rat:Treatment, 6;
Treatment, 3
```

```
Fixed effects:
               Estimate  Std. Error  t value
(Intercept)     140.500       5.184   27.104
Treatment2       10.500       7.331    1.432
Treatment3       -5.333       7.331   -0.728

Correlation of Fixed Effects:
             (Intr)  Trtmn2
Treatment2   -0.707
Treatment3   -0.707   0.500
```

You can see that the treatment effect is correctly interpreted as being non-significant ($t < 2$). The variance components (p. 478) can be extracted by squaring the standard deviations, then expressing them as percentages:

```
vars<- c(14.1617,36.0843,21.1678)
100*vars/sum(vars)
```

```
[1] 19.83048 50.52847 29.64105
```

so 50.5% 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 (see p. 333). You can extract the variance components with the VarCorr(model) function.

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. 629):

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

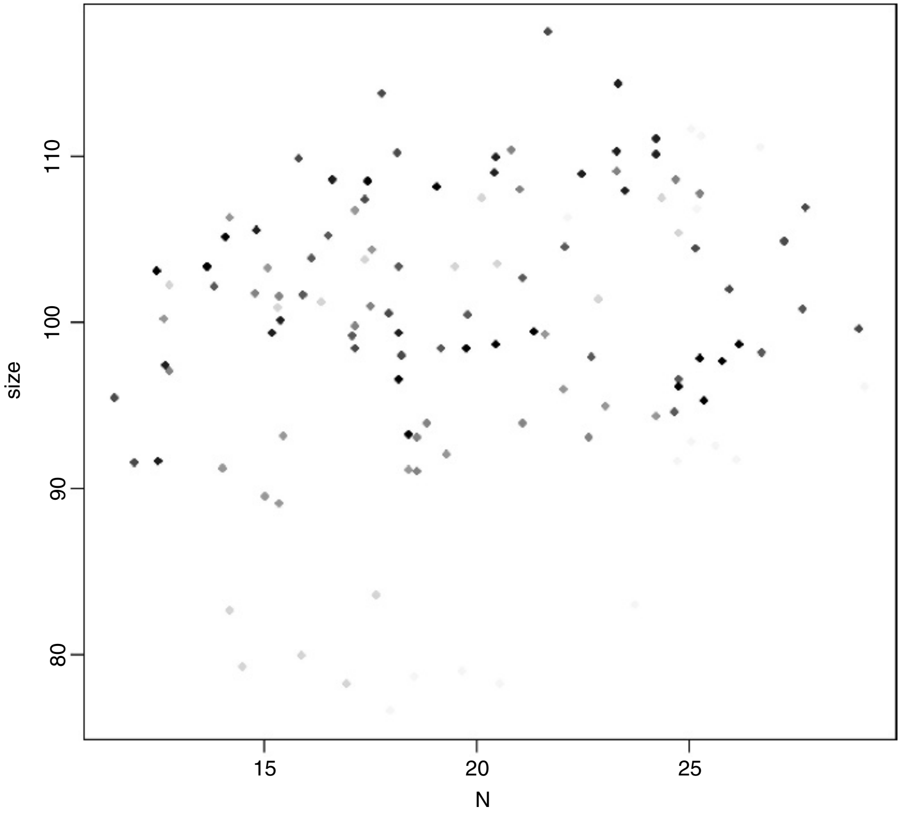
```
[1] "N"  "size"  "farm"
```

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

```
plot(N,size,pch=16,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 (yellow) fields have a mean *y* value of less than 80, while the maximum (red) fields have a mean *y* value above 110.

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 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. 398); 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:

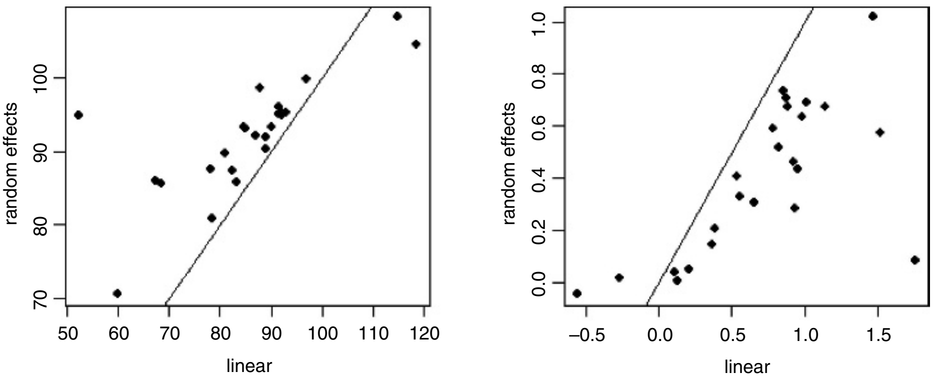
```
random.model<-lme(size~1,random=~N|farm)
coef(random.model)
```

```
    (Intercept)            N
1      85.98139  0.574205307
2     104.67366 -0.045401473
3      95.03442  0.331080922
4      98.62679  0.463579823
5      95.00270  0.407906211
6      99.82294  0.207203698
7      85.57345  0.285520353
8      96.09461  0.520896471
9      95.22186  0.672262924
10     93.14157  1.017995727
11    108.27200  0.015213748
12     87.36387  0.689406409
13     80.83933  0.003617002
14     89.84309  0.306402249
15     93.37050  0.636778709
16     92.10914  0.145772153
17     94.93395  0.084935465
18     85.90160  0.709943262
19     92.00628  0.052485986
20     95.26296  0.738029400
21     93.35069  0.591151955
22     87.66161  0.673119269
23     70.57827  0.432993915
24     90.29151  0.036747120
```

Variation in the intercepts explains 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. 631), and is most clear from a graphical comparison of the coefficients of the linear and mixed models:

```
mm<-coef(random.model)
ll<-coef(linear.models)
```

```
par(mfrow=c(2,2))
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, and 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 ($p < 0.0001$).

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
-3.60765  -1.29473  0.04789  1.07322  4.12972
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’s 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     -72.21  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.

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 function glmmPQL 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, as explained in Chapter 14. 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, 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. 604–609. 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 were made is also recorded.

Fixed Effects in Hierarchical Sampling

Given that the gender effect in our hierarchical sampling example on p. 639 was so large, and that gender makes a sensible fixed effect (it has informative factor levels: male and female), we might fit gender as a main effect. The important point to note is that when you want to compare models with different fixed effects using lme you must change the fitting method from the default REML to the optional maximum likelihood method="ML". This then allows you to use anova to compare lme models with different fixed effects:

```
model10<-lme(subject~gender,random=~1|town/district/street/family/gender,
                                        method="ML")
model11<-lme(subject~1,random=~1|town/district/street/family/gender,
                                        method="ML")
anova(model10,model11)
```

```
        Model  df       AIC       BIC     logLik    Test   L.Ratio  p-value
model10     1   8  3331.584  3368.218  -1657.792
model11     2   7  3352.221  3384.276  -1669.111  1 vs 2  22.63755   <.0001
```

It is clear that the model with gender as a fixed effect (model10) is vastly superior to the model with out any fixed effects ($p < 0.0001$). It has a much lower AIC, despite its extra parameter. The variance components have been little affected by fitting gender as a fixed effect, and the effect size of gender is given by:

```
summary(model10)
```

```
Fixed effects: subject ~ gender
                  Value  Std.Error   DF    t-value  p-value
(Intercept)    8.976328  0.6332402  360  14.175234        0
gendermale    -1.930773  0.3936610  179  -4.904659        0
```

You can see what the parameter values are by looking at the treatment means:

```
tapply(subject,gender,mean)
```

```
  female      male
8.976328  7.045555
```

The intercept in the lme is the mean for females and the gender.male effect is the difference between the male and female means: $7.045\,555 - 8.976\,328 = -1.930\,773$.

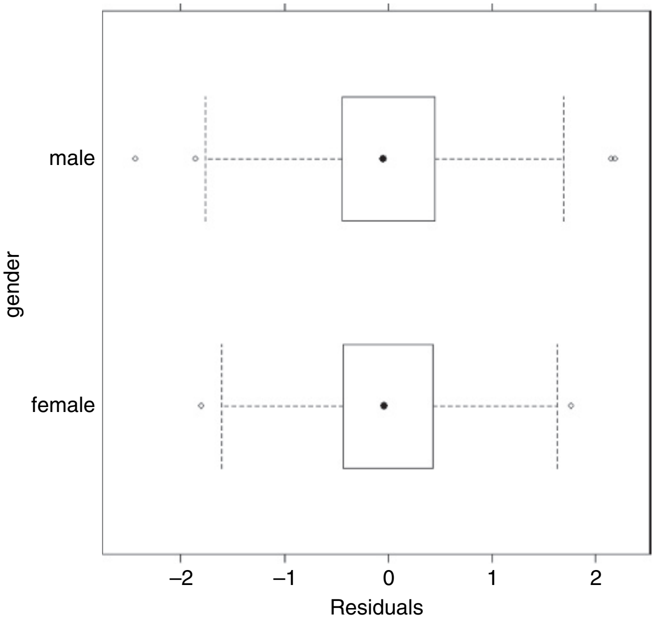
Error Plots from a Hierarchical Analysis

You will find the syntax of model checking in lme both complicated and difficult to remember.

```
library(nlme)
library(lattice)
trellis.par.set(col.whitebg())
```

If you use the standard plot(model) with lme you get a single panel showing the standardized residuals as a function of the fitted values. For more comprehensive model checking, it is useful to make the dataframe into a groupedData object, then refit the model. Here we investigate the REML model with gender as a fixed effect:

```
hs<-groupedData(subject~gender|town/district/street/family/gender/replicate,
                                              outer=~gender,data=hierarchy)
model<-
lme(subject~gender,random=~1|town/district/street/family/gender,data=hs)
plot(model,gender~resid(.))
```



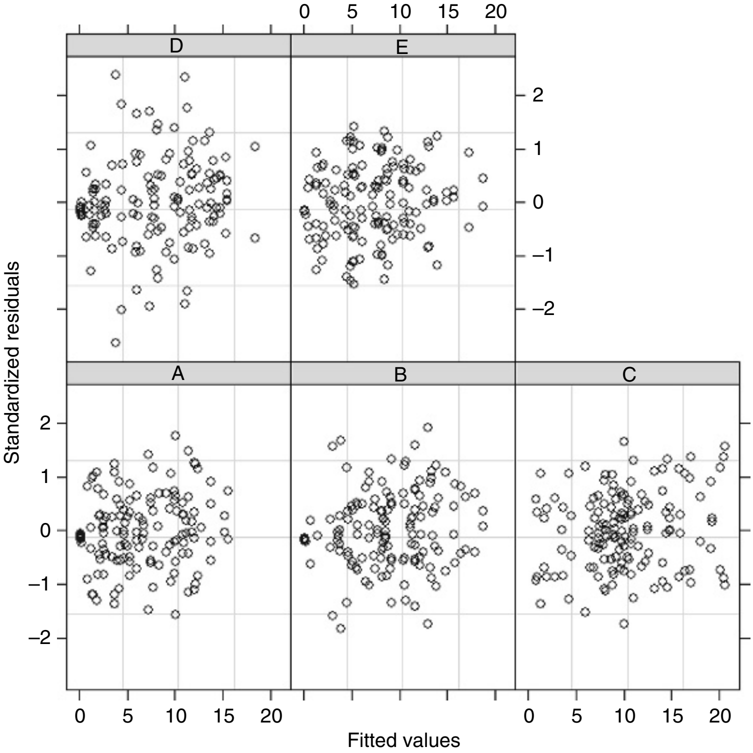
To inspect the constancy of variance across towns and check for heteroscedasticity:

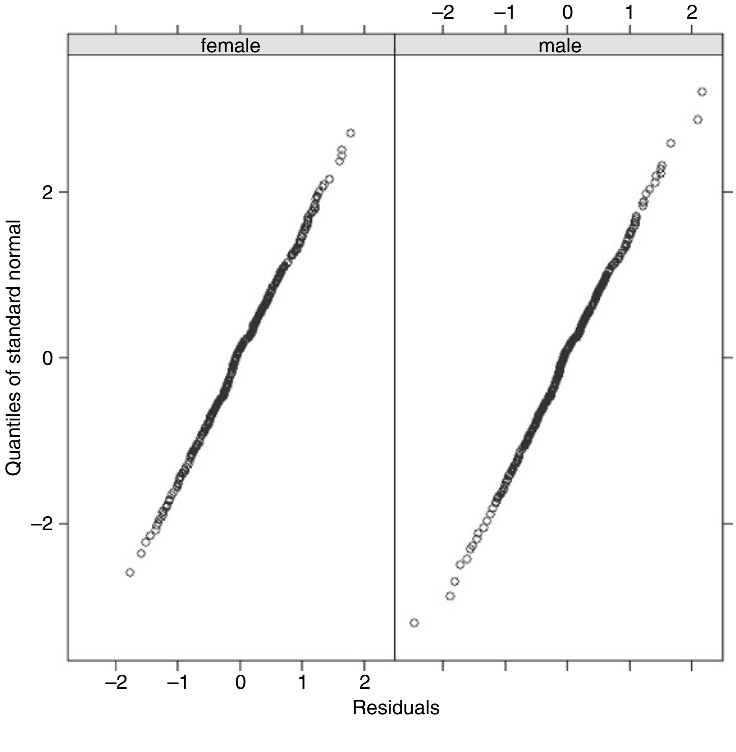
```
plot(model,resid(.,type="p")~fitted(.)|town)
```

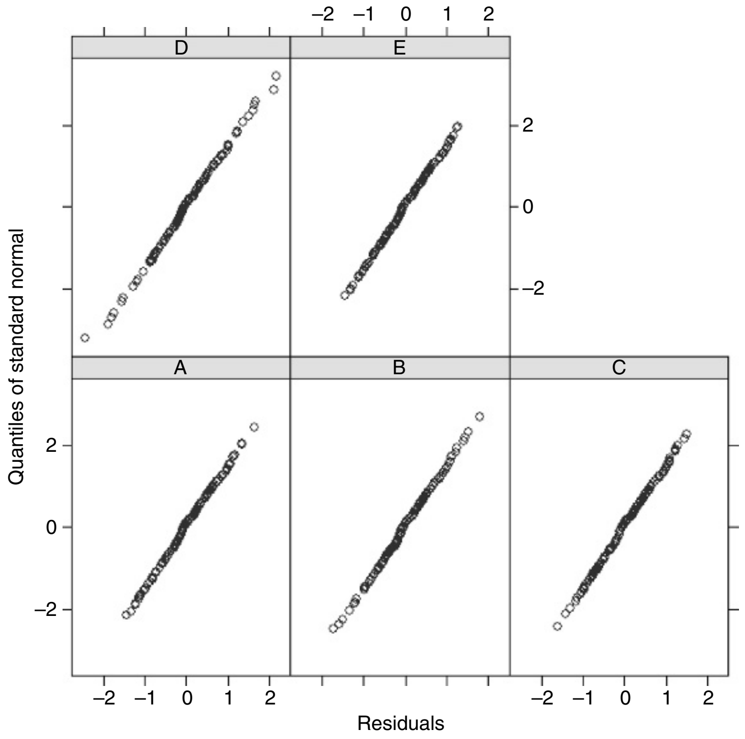
It should be clear that this kind of plot only makes sense for those variables with informative factor levels such as gender and town; it would make no sense to group together the streets labelled s1 or s3 or the families labelled f1, f2 or f3.

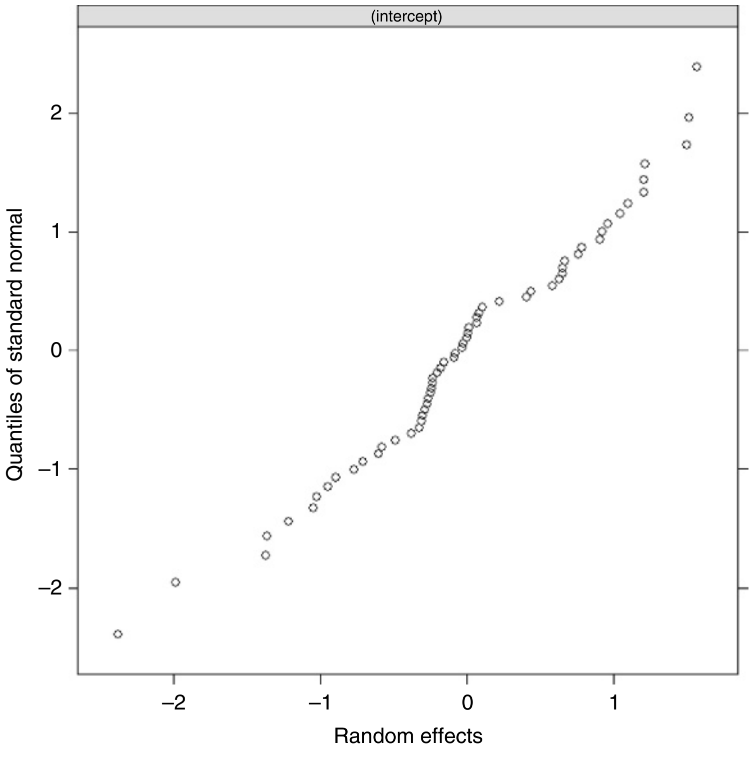
Tests for normality use the familiar QQ plots, but applied to panels:

```
qqnorm(model,~resid(.)|gender)
```


D
E
A
B
C
Standardized residuals
Fitted values
0
5
10
15
20
2
1
0
−1
−2


female
male
Quantiles of standard normal
Residuals
−2
−1
0
1
2


D
E
A
B
C
–2
–1
0
1
2
Quantiles of standard normal
Residuals


(intercept)
2
1
0
–1
–2
Quantiles of standard normal
Random effects

The residuals are normally distributed for both genders, and within each town:

```
qqnorm(model,~resid(.)|town)
```

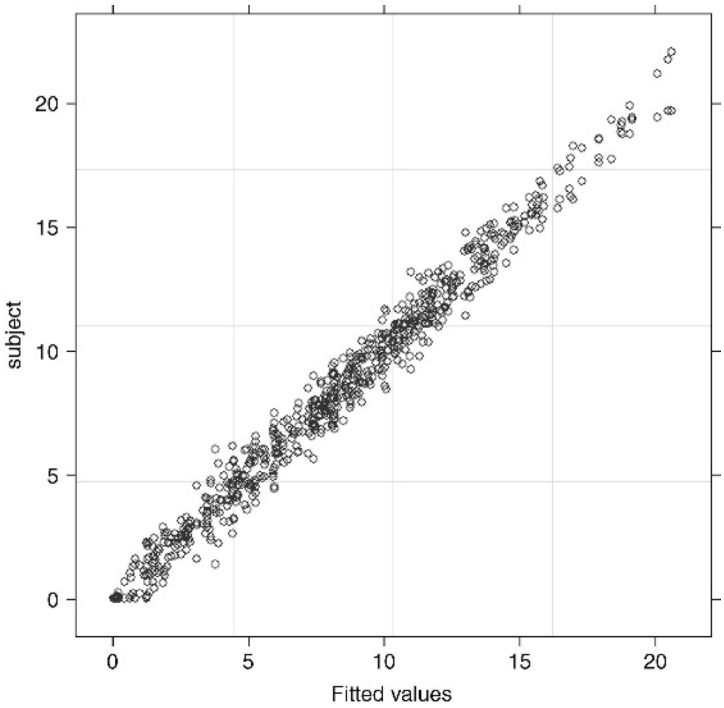
To assess the normality of the random effects, you need to specify the level of interest. In this example we have five levels, and you can experiment with the others:

```
qqnorm(model,~ranef(.,level=3))
```

By level 5, the random effects are beautifully normal, but at level 1 (towns) the data are too sparse to be particularly informative. Here at level 3 there is reasonable, but not perfect, normality of the random effects.

Finally, we plot the response variable plotted against the fitted values:

```
plot(model,subject~fitted(.))
```


subject
Fitted values