9

Analysis of Variance

Analysis of variance is the technique we use when all the explanatory variables are categorical. The explanatory variables are called **factors**, and each factor has two or more **levels**. When there is a single factor with three or more levels we use one-way Anova. If we had a single factor with just two levels, we would use Student's test (see p. 76), and this would give us exactly the same answer that we would have obtained by Anova (remember the rule that $F = t^2$). Where there are two or more factors, then we use two-way or three-way Anova, depending on the number of explanatory variables. When there is replication at each level in a multi-way Anova, the experiment is called a **factorial** design, and this allows us to study **interactions** between variables, in which we test whether the response to one factor depends on the level of another factor.

One-way Anova

There is a real paradox about analysis of variance, which often stands in the way of a clear understanding of exactly what is going on. The idea of analysis of variance is to compare two or more means, but it does this by comparing variances. How can that work?

The best way to see what is happening is to work through a graphical example. To keep things as simple as possible, we shall use a factor with just two levels at this stage, but the argument extends to any number of levels. Suppose that we have atmospheric ozone concentrations measured in parts per hundred million (pphm) in two commercial lettuce-growing gardens (we shall call the gardens A and B for simplicity).

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

```
[ 1]  "ozone"    "garden"
```

As usual, we begin by plotting the data, but here we plot the y values (ozone concentrations) against the order in which they were measured:

```
plot(1:20,ozone,ylim = c(0,8),ylab = "y",xlab = "order")
```

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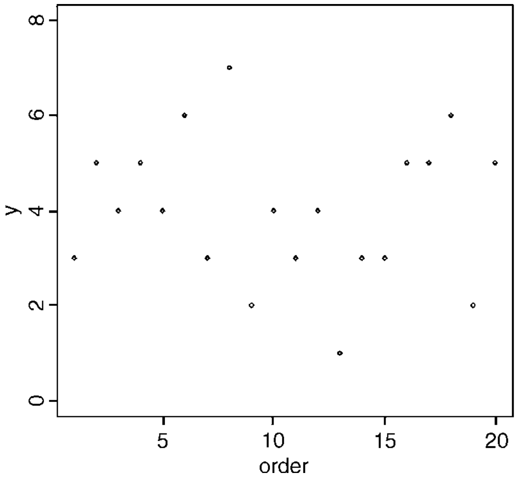
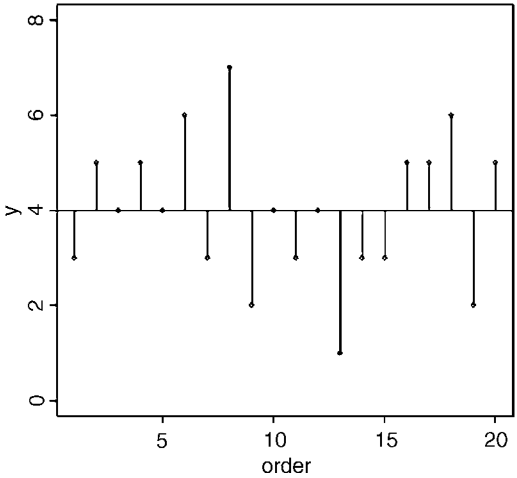


There is lots of scatter, indicating that the variance in y is large. To get a feel for the overall variance, we can plot the mean value of y and indicate each of the residuals by a line from mean(y) to the value of y:

```
abline(mean(ozone),0)
for(i in 1:20) lines(c(i,i),c(mean(ozone),ozone[i]))
```



We refer to this overall variation as the **total sum of squares**, *SSY*, which more formally is given by:

$$SSY = \sum (y - \bar{y})^2$$

which should look familiar, because it is the formula used in defining the variance of y ($s^2 =$ sum of squares/degrees of freedom; see p. 37).

This next step is the key to understanding how analysis of variance works. Instead of fitting the overall mean value of *y* through the data, and looking at the departures of all the data points from the overall mean, let's fit the individual treatment means (the mean for garden A and the mean for garden B in this case), and look at the departures of data points from the appropriate treatment mean. It will be useful if we have different plotting symbols for the different gardens; say open circles (pch = 1) for garden A and solid circles (pch = 16) for garden B. Note the type of type = "n" to suppress plotting when we first draw the axes:

```
plot(1:20,ozone,ylim = c(0,8),type = "n",ylab = "y",xlab = "order")
```

Now add the points for garden A:

```
points(seq(1,19,2),ozone[garden = = "A"],pch = 1)
```

To space out the points, we put data from the two gardens in alternating positions on the graph, using seq(1,19,2) for garden A and seq(2,20,2) for garden B:

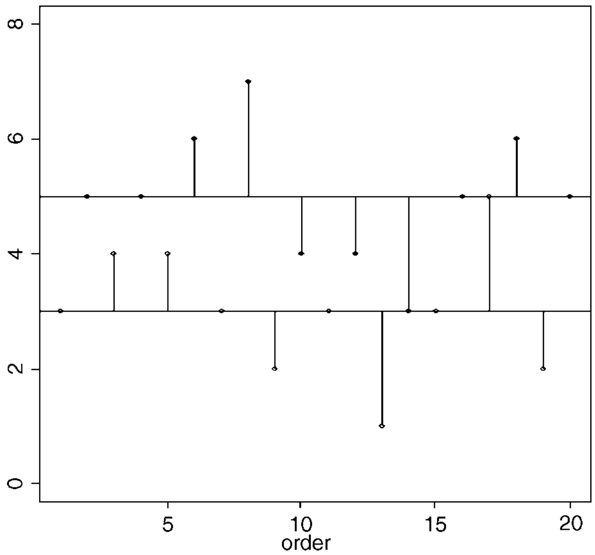
```
points(seq(2,20,2),ozone[garden = = "B"],pch = 16)
```

Now it is clear that the mean ozone concentration in garden B is substantially higher. The aim of analysis of variance is to determine whether it is significantly higher, or whether this kind of difference could come about by chance alone, when the mean ozone concentrations in the two gardens was really the same.

Now we draw the residuals–the differences between the measured ozone concentrations, and the means for the gardens involved:

```
abline(mean(ozone[garden = = "A"]),0)
abline(mean(ozone[garden = = "B"]),0)

k < - -1
for (i in 1:10){
k < -k+2
lines(c(k,k),c(mean(ozone[garden= = "A"]),ozone[garden= = "A"] [i]))
lines(c(k+1,k+1),c(mean(ozone[garden=="B"]),ozone[garden= = "B"] [i]))
}
```

This raises some questions. If the means in the two gardens are not significantly different, what should be the difference in the lengths of the residual lines in this figure and the figure before? After a bit of thought, you should see that if the means were the same, then the two horizontal lines in this figure would be in the same place, and hence the lengths of the residual lines would be the same as in the previous figure. We're half way there. Now, suppose that mean ozone concentration is different in the two gardens. Would the residual lines be bigger or smaller when we compute them from the individual treatment means (as above), or from the overall mean (as in the previous figure)? They would be **smaller** when computed from the individual treatment means **if the individual treatment means were different**.



So there it is. That is how analysis of variance works. **When the means are significantly different, then the sum of squares computed from the individual treatment means will be smaller than the sum of squares computed from the overall mean**. We judge the significance of the difference between the two sums of squares using analysis of variance.

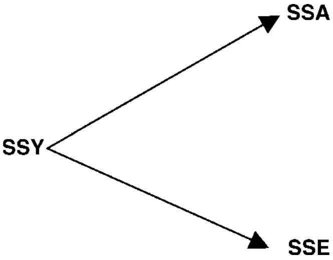
The analysis is formalized by defining this new sum of squares: it is the sum of the squares of the differences between the individual y values and the relevant treatment mean. We shall call this *SSE*, the **error sum of squares** (there has been no error in the sense of a mistake; 'error' is used here as a synonym of 'residual'):

$$SSE = \sum_{j=1}^{k} \sum (y - \bar{y}_j)^2.$$

We compute the mean for the jth level of the factor in advance, and then add up the squares of the differences. Given that we worked it out this way, can you see how many degrees of freedom should be associated with *SSE*? Suppose that there were n replicates in each treatment ($n = 10$ in our example). And suppose that there are k levels of the factor ($k = 2$ in our example). If you estimate k parameters from the data before you can work out *SSE*, then you must have lost k degrees of freedom in the process. Since each of the k levels of the factor has n replicates, there must be $k \times n$ numbers in the whole experiment ($2 \times 10 = 20$ in our example). So the degrees of freedom associated with *SSE* is $k.n - k = k(n - 1)$. Another way of seeing this is to say that there are n replicates in each treatment, and hence $n - 1$ degrees of freedom for error in each treatment (because 1 d.f. is lost in estimating each treatment mean). There are k treatments (i.e. k levels of the factor) and hence there are $k \times (n - 1)$ d.f. for error in the experiment as a whole.

Now we come to the 'analysis' part of the analysis of variance. The total sum of squares in y, *SSY*, is broken up (analysed) into components. The unexplained part of the variation is called the error sum of squares, *SSE*. The component of the variation that is explained by differences between the treatment means is called the treatment sum of squares, and is traditionally denoted by *SSA*. This is because in two-way analysis of variance, with two different categorical explanatory variables, we shall use *SSB* to denote the sum of squares attributable to differences between the means of the second factor, *SSC* to denote the sum of squares attributable to differences between the means of the third factor, and so on.

Analysis of variance, therefore, is based on the notion that we break down the total sum of squares, *SSY*, into useful and informative components:



Typically, we compute all but one of the components, then find the value of the last component by subtraction of the others from *SSY*. We already have a formula for *SSE*, so we could obtain *SSA* by difference: $SSA = SSY - SSE$. Starting with *SSY* we calculate the sum of the squares of the differences between the y values and the overall mean:

```
SSY < -sum((ozone-mean(ozone))^2)
SSY
```

```
[ 1] 44
```

The question now is 'how much of this 44 is attributable to differences between the means of gardens A and B (SSA = explained variation) and how much is sampling error (SSE = unexplained variation)?'. We have a formula defining *SSE*; it is the sum of the squares of the residuals calculated separately for each garden, using the appropriate mean value. For garden A we get

```
sum((ozone[garden == "A"]-mean(ozone[garden == "A"]))^2)
```

```
[ 1] 12
```

and for garden B

```
sum((ozone[garden == "B"]-mean(ozone[garden == "B"]))^2)
```

```
[ 1] 12
```

so the error sum of squares is the total of these components $SSE = 12 + 12 = 24$. Finally, we can obtain the treatment sum of squares, *SSA*, by difference: $SSA = 44 - 24 = 20$.

At this point, we can fill in the Anova table (see p. 136):

Source	Sum of squares	Degrees of freedom	Mean square	F-ratio
Garden	20.0	1	20.0	15.0
Error	24.0	18	$s^2 = 1.3333$	
Total	44.0	19		

We need to test whether an F-ratio of 15.0 is large or small. To do this we compare it with the critical value of F from quantiles of the F-distribution, qf. We have one degree of freedom in the numerator, and 18 degrees of freedom in the denominator, and we want to work at 95% certainty ($\alpha = 0.05$):

```
qf(0.95,1,18)
```

```
[1] 4.413873
```

The calculated value of 15.0 is much greater than the critical value of $F = 4.41$, so we can reject the null hypothesis (equality of the means) and accept the alternative hypothesis (the two means are significantly different). We used a one-tailed F-test (0.95 rather than 0.975 in the qf function) because we are only interested in the case where the treatment variance is large relative to the error variance. This approach is rather old-fashioned; the modern view is to calculate the **effect size** (the difference between the means is 2.0 pphm ozone) and to state the probability that such a difference would arise by chance alone when the difference between the means was actually 0. For this we use cumulative probabilities of the F distribution, rather than quantiles, like this:

```
1-pf(15.0,1,18)
```

```
[1] 0.001114539
```

So the probability of obtaining data as extreme as ours (or more extreme) if the two means really were the same is roughly one tenth of 1%.

That was quite a lot of work. Here is the whole analysis in R in a single line:

```
summary(aov(ozone~garden))
```

```
             Df   Sum Sq   Mean Sq  F value      Pr(>F)
garden        1  20.0000   20.0000       15   0.001115**
Residuals    18  24.0000    1.3333
```

The first column shows the sources of variation (*SSA* and *SSE* respectively); note that R leaves off the row that we had included for total variation, *SSY.* The next column shows degrees of freedom: there are two levels of garden (A and B) so there is $2 - 1 = 1$ d.f. for garden, and there are 10 replicates per garden, so $10 - 1 = 9$ d.f. per garden and two gardens, so error d.f. $= 2 \times 9 = 18$. The next column shows the sums of squares: $SSA = 20$ and $SSE = 24$. The fourth column gives the mean squares (sums of squares divided by degrees of freedom); the treatment mean square is 20.0 and the error variance, s^2 (synonym of the residual mean square) is 1.3333. The F ratio is 15, and the probability that this (or a more extreme result) would arise by chance alone if the two means really were the same, is 0.001115 (as we calculated long-hand, above).

We finish by carrying out graphical checks of the assumptions of the model, namely constancy of variance and normality of errors.

```
plot(aov(ozone~garden))
```

The first plot on your screen shows that the variances are identical in the two treatments (this is exactly what we want to see). The second plot shows a reasonably straight-line relationship on the Normal quantile–quantile plot (especially since, in this example, the y values are whole numbers), so we can be confident that non-normality of errors is not a major problem. The third plot shows the residuals against the fitted values on a different scale, and the fourth plot shows Cook's statistics, drawing attention to the fact that points 8, 13 and 14 are potentially influential. We can test for their influence by repeating the analysis but leaving out these points:

```
wanted=(1:20 !=8 & 1:20 !=13 & 1:20 !=14)
wanted
```

```
[ 1]  TRUE TRUE TRUE TRUE TRUE TRUE TRUE FALSE TRUE TRUE TRUE TRUE
[ 13]  FALSE FALSE TRUE TRUE TRUE TRUE TRUE TRUE
```

We can use subset to leave out the three potentially influential points:

```
summary(aov(ozone~garden,subset=wanted))
```

```
             Df    Sum Sq    Mean Sq   F value       Pr(>F)
garden        1   13.3856    13.3856    17.376   0.0008239***
Residuals    15   11.5556     0.7704
```

The interpretation is unaffected; only the degrees of freedom (15 instead of 18 d.f. for error) and the p value have changed.

Shortcut Formula

In the unlikely event that you ever need to do analysis of variance using a calculator, then it is useful to know the shortcut formula for calculating SSA. We calculated it by difference, above, having worked out SSE longhand. To do this, the thing you need to understand is what we mean by a 'treatment total'. The treatment total is simply the sum of the y values in a particular factor level. For our two gardens we have:

```
cbind(ozone[garden == "A"],ozone[garden == "B"])
```

```
      [ ,1] [ ,2]
 [ 1,]    3    5
 [ 2,]    4    5
 [ 3,]    4    6
 [ 4,]    3    7
 [ 5,]    2    4
 [ 6,]    3    4
 [ 7,]    1    3
 [ 8,]    3    5
 [ 9,]    5    6
[ 10,]    2    5
```

```
tapply(ozone,garden,sum)
```

```
 A      B
30     50
```

The totals for gardens A and B are 30 and 50 respectively, and we shall call these T_1 and T_2. The shortcut formula for *SSA* (Box 9.1) is then:

$$SSA = \frac{\sum T_i^2}{n} - \frac{\left(\sum y\right)^2}{kn}.$$

We should check that this really does give the correct value for *SSA*:

$$SSA = \frac{30^2 + 50^2}{10} - \frac{80^2}{2 \times 10} = \frac{3400}{10} - \frac{6400}{20} = 340 - 320 = 20$$

which checks out. In all sorts of analysis of variance, the key point to realize is that the sum of the subtotals squared is always **divided by the number of numbers that were added together to get each subtotal**. That sounds complicated, but the idea is simple. In our case we squared the subtotals T_1 and T_2 and added the results together. We divided by 10 because both T_1 and T_2 were the sum of ten numbers.

Box 9.1. Corrected sums of squares in one-way Anova

The definition of the total sum of squares, *SSY*, is the sum of the squares of the differences between the data points, *y*, and the overall mean, $\bar{\bar{y}}$

$$SSY = \sum_{i=1}^{k} \sum (y - \bar{\bar{y}})^2$$

where $\sum$ means the sum over the n replicates within each of the k factor levels. the error sum of squares, *SSE*, is the sum of the squares of the differences between the data points, y, and their individual treatment means, $\bar{y}_i$

$$SSE = \sum_{i=1}^{k} \sum (y - \bar{y}_i)^2.$$

The treatment sum of squares, *SSA*, is the sum of the squares of the differences between the individual treatment means, $\bar{y}_i$, and the overall mean, $\bar{\bar{y}}$

$$SSA = \sum_{i=1}^{k} \sum_{j=1}^{n} (\bar{y}_i - \bar{\bar{y}})^2 = n \sum_{i=1}^{k} (\bar{y}_i - \bar{\bar{y}})^2.$$

Squaring the bracketed term, and applying summation gives

$$\sum \bar{y}_i^2 - 2\bar{\bar{y}} \sum \bar{y}_i + k.\bar{\bar{y}}^2.$$

Now replace $\bar{y}_i$ by T_i/n (where T is our conventional name for the k individual treatment totals) and replace $\bar{\bar{y}}$ by $\sum y/k.n$ to get

$$\frac{\sum_{i=1}^{k} T_i^2}{n^2} - 2\frac{\sum y \sum_{i=1}^{k} T_i}{n.k.n} + k\frac{\sum y \sum y}{k.n.k.n.}.$$

Note that $\sum_{i=1}^{k} T_i = \sum_{i=1}^{j} \sum_{j=1}^{n} y_{ij}$ so the right-hand positive and negative terms both have the form $(\sum y)^2/k.n^2$. Finally, multiplying through by n gives

$$SSA = \frac{\sum T^2}{n} - \frac{(\sum y)^2}{k.n.}.$$

As an exercise, you should prove that $SSY = SSA + SSE$ (and see Box 8.5).

Effect Sizes

So far we have concentrated on hypothesis testing, using summary.aov. It is usually more informative to investigate the effects of the different factor levels, using summary.lm like this:

```
summary.lm(aov(ozone~garden))
```

It was easy to interpret this kind of output in the context of a regression, where the rows represent parameters that are intuitive–namely, the intercept and the slope. In the context of analysis of variance, it takes a fair bit of practice before the meaning of this kind of output is transparent.

```
Coefficients:
                Estimate   Std. Error   t value   Pr(>|t|)
(Intercept)       3.0000       0.3651     8.216   1.67e-07 ***
gardenB           2.0000       0.5164     3.873    0.00111 **

Residual standard error:  1.155 on 18 degrees of freedom
Multiple R-Squared: 0.4545,    Adjusted R-squared:  0.4242
F-statistic:        15 on 1 and 18 DF,    p-value: 0.001115
```

The rows are labelled (Intercept) and gardenB, but what do the parameter estimates 3.0 and 2.0 actually mean? Why are the standard errors different in the two rows? After all, the variances in the two gardens were identical.

To understand the answers to these questions, we need to know how the equation for the explanatory variables is structured when the explanatory variable, as here, is categorical. To recap, the linear regression model is written as

```
lm(y~x)
```

which R interprets as the two-parameter linear equation

$$y = a + bx$$

in which the values of the parameters a and b are to be estimated from the data. But what about our analysis of variance? We have one explanatory variable, $x =$ 'garden', with two levels, 'A' and 'B'. The aov model is exactly analogous to the regression model

```
aov(y~x)
```

but what is the associated equation? Let's look at the equation first, then try to understand it:

$$y = a + bx_1 + cx_2.$$

This looks just like a multiple regression, with two explanatory variables, x_1 and x_2. The key point to understand is that x_1 and x_2 are **the levels of the factor called x**. If 'garden' was a four-level factor, then the equation would have four explanatory variables in it, x_1 to x_4. With a categorical explanatory variable, the levels are all coded as 0 except for the level associated with the y value in question, which is coded as 1. You will find this hard to understand without a good deal of practice. Let's look at the first row of data in our dataframe:

```
garden[1]
```

```
[1] A
```

So the first ozone value in the dataframe comes from garden A. This means that $x_1 = 1$ and $x_2 = 0$. The equation for the first row therefore looks like this:

$$y = a + b \times 1 + c \times 0 = a + b \times 1 = a + b.$$

What about the second row of the dataframe?

```
garden[2]
```

```
[ 1] B
```

Because this row refers to garden B, x_1 is coded as 0 and x_2 is coded as 1 so the equation becomes

$$y = a + b \times 0 + c \times 1 = a + c \times 1 = a + c.$$

So what does this tell us about the parameters *a, b* and *c*? And why do we have three parameters, when the experiment generates only two means? These are the crucial questions for understanding the summary.lm output from an analysis of variance. The simplest interpretation of the three-parameter case that we have dealt with so far is that the (Intercept) *a* is the overall mean from the experiment:

```
mean(ozone)
```

```
[ 1] 4
```

Then, if *a* is the overall mean, so $a + b$ must be the mean for garden A and $a+c$ must be the mean for garden B (see the equations, above). If that is true, then *b* must be **the difference between the mean of garden A and the overall mean**. And *c* must be **the difference between the mean of garden B and the overall mean**. Thus, the (Intercept) is a **mean**, and the other parameters are **differences between means**. This explains why the standard errors are different in the different rows of the table: the standard error of the intercept is the standard error of a mean

$$\text{s.e.}_{\bar{y}} = \sqrt{\frac{s_A^2}{n_A}},$$

whereas the standard errors on the other rows are standard errors of the difference between two means:

$$\text{s.e.}_{\text{diff}} = \sqrt{\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}}$$

which is a bigger number (bigger by a factor of $1.4142 = \sqrt{2}$ if, as here, the sample sizes and variances are equal).

With three parameters, then, we should have $b =$ mean ozone concentration in garden A − 4 and $c =$ mean ozone concentration in garden B − 4.

```
mean(ozone[garden=="A"])-mean(ozone)
```

```
[1] -1
```

```
mean(ozone[garden=="B"])-mean(ozone)
```

```
[1]  1
```

That would be a perfectly reasonable way to parameterize the model for this analysis of variance, but it suffers from the fact that there is a redundant parameter. The experiment produces only two means (one for each garden), and so there is no point in having three parameters to represent the output of the experiment. One of the three parameters is said to be ‘aliased’. There are lots of ways round this dilemma, as explained in detail in Chapter 12 on Contrasts. Here we adopt the convention that is used as the default in R: so called **treatment contrasts**. Under this convention, we dispense with the overall mean, *a*. So now we are left with the right number of parameters (*b* and *c*). In treatment contrasts, **the factor level that comes first in the alphabet is set equal to the Intercept**. The other parameters are expressed as differences between this mean and the other relevant means. So, in our case, the mean of garden A becomes the intercept

```
mean(ozone[garden= ="A"])
```

```
[1]  3
```

and the difference between the means of garden B and garden A is the second parameter:

```
mean(ozone[garden=="B"])-mean(ozone[garden=="A"])
```

```
[1] 2
```

Let’s revisit our summary.lm table and see if it now makes sense:

```
Coefficients:
              Estimate  Std. Error  t value   Pr(>|t|)
(Intercept)     3.0000      0.3651    8.216   1.67e-07   ***
gardenB         2.0000      0.5164    3.873    0.00111   **
```

The (Intercept) is 3.0 which is the mean for garden A (because the factor level ‘A’ comes before level ‘B’ in the alphabet). The estimate for garden B is 2.0. This tells us that the mean ozone concentration in garden B is 2 p.p.h.m. greater than in garden A (greater because there is no minus sign–absence of a sign implies ‘plus’). We would compute the mean for garden B as $3.0 + 2.0 = 5.0$. In practice, we would not obtain the means like this, but by using tapply, instead:

```
tapply(ozone, garden, mean)
```

```
A  B
3  5
```

There is more about these issues in Chapter 12.

Plots for Interpreting One-way Anova

There are two traditional ways of plotting the results of Anova:

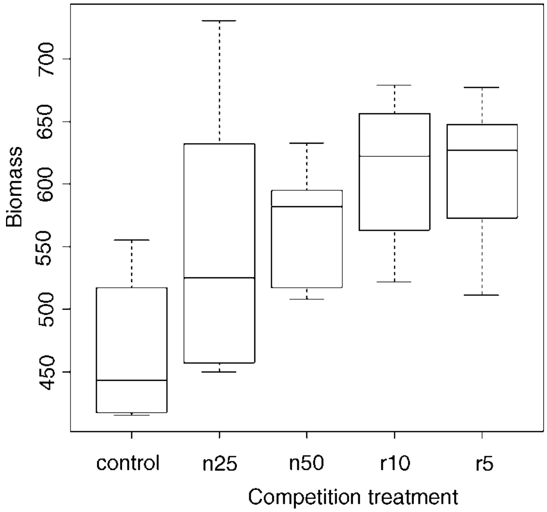
- box and whisker plots, and
- bar plots with error bars.

Here is an example to compare the two approaches. We have an experiment on plant competition with one factor and five levels. The factor is called clipping and the levels are control (i.e. unclipped) with two intensities of shoot pruning and two intensities of root pruning:

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

```
[ 1] "biomass" "clipping"
```

```
plot(clipping,biomass,xlab="Competition treatment",ylab="Biomass")
```



The box and whisker plot is good at showing the nature of the variation within each treatment, and also whether there is skew within each treatment (e.g. for the control plots, there is a wider range of values in the 50%–75% quartile than in the 25%–50% quartile). No outliers are shown above the whiskers, so the tops and bottoms of the bars are the maxima and minima within each treatment. The medians for the competition treatments are all higher than the 75% percentile of the controls, suggesting that they may be significantly different from the controls, but there is little to suggest that any of the competition treatments are significantly different from one another (see below for the analysis). We could use the notch=T option to get a visual impression of the significance of differences between the means; all the treatment medians fall outside the notch of the controls, but no other comparisons appear to be significant.

Barplots with error bars are preferred by many journal editors, and some people think that they make hypothesis testing easier. We shall see. Unlike S-Plus, R does not have a built-in function called error.bar so we shall have to write our own. Here is a very simple version without any bells or whistles. We shall call it error.bars to distinguish it from the much more general S-Plus function.

```
error.bars < -function(yv,z,nn){
xv < -
barplot(yv,ylim = c(0,(max(yv) + max(z))),names = nn,
   ylab = deparse(substitute(yv)))
g<- (max(xv)-min(xv))/50
for (i in 1:length(xv)) {
lines(c(xv[i],xv[i]),c(yv[i] + z[i],yv[i]-z[i]))
lines(c(xv[i]-g,xv[i] + g),c(yv[i] + z[i], yv[i] + z[i]))
lines(c(xv[i]-g,xv[i] + g),c(yv[i]-z[i], yv[i]-z[i]))
}}
```

To use this function we need to decide what kind of values (z) to use for the lengths of the bars. Let's use the standard error of a mean based on the pooled error variance from the Anova, then return to a discussion of the pros and cons of different kinds of error bars later. Here is the one-way analysis of variance:

```
model < -aov(biomass ~ clipping)
summary(model)
```

```
             Df   Sum Sq  Mean Sq  F value    Pr(>F)
clipping      4    85356    21339   4.3015  0.008752  **
Residuals    25   124020     4961
```

From the Anova table we learn that the pooled error variance $s^2 = 4961.0$. Now we need to know how many numbers were used in the calculation of each of the five means:

```
table(clipping)
```

```
clipping
control   n25   n50  r10    r5
      6     6     6    6     6
```

There was equal replication (which makes life easier), and each mean was based on six replicates, so the standard error of a mean is $\sqrt{s^2/n} = \sqrt{4961/6} = 28.75$. We shall draw an error bar up 28.75 from each mean and down by the same distance, so we need five values for z, one for each bar, each of 28.75:

```
se < -rep(28.75,5)
```

We need to provide labels for the five different bars – the factor levels should be good for this:

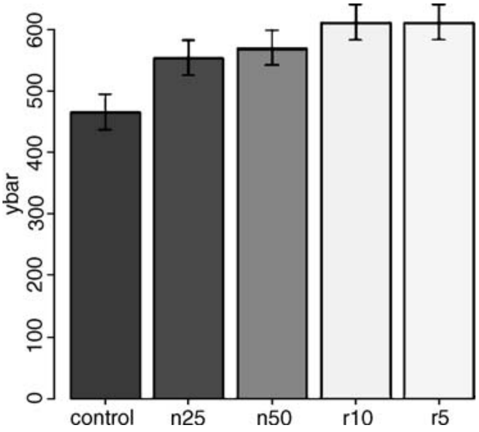
```
labels < -as.character(levels(clipping))
```

Now we work out the five mean values which will be the heights of the bars, and save them as a vector called ybar:

```
ybar < -as.vector(tapply(biomass,clipping,mean))
```

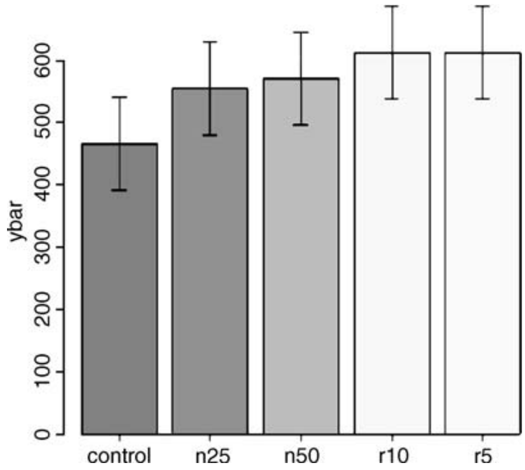
Now we can create the barplot with error bars:

```
error.bars(ybar,se,labels)
```



We do not get the same feel for the distribution of the values within each treatment as was obtained by the box and whisker plot, but we can certainly see clearly which means are not significantly different. If, as here, we use ±1 s.e. as the length of the error bars, then **when the bars overlap this implies that the two means are not significantly different**. Remember the rule of thumb for *t*: significance requires two or more standard errors, and if the bars overlap it means that the difference between the means is less than two standard errors. There is another issue, too. For comparing means, we should use the standard error of the difference between two means (not the standard error of one mean) in our tests (see p. 165); these bars would be about 1.4 times as long as the bars we have drawn here. So while we can be sure that the two root-pruning treatments are not significantly different from one another, and that the two shoot-pruning treatments are not significantly different from one another (because their bars overlap), we cannot conclude from this plot that the controls have significantly lower biomass than the rest (because the error bars are not the correct length for testing differences between means).

An alternative graphical method is to use 95% confidence intervals for the lengths of the bars, rather than standard errors of means. This is easy to do: we multiply our standard errors by Student's t qt(.975,5) $= 2.570582$ to get the lengths of the confidence intervals:



Now, all of the error bars overlap, implying visually that there are no significant differences between the means. However, we know that this is not true from our analysis of variance, in which we rejected the null hypothesis that all the means were the same at $p = 0.00875$. If it were the case that the bars did not overlap when we are using confidence intervals (as here), then that would imply that the means differed by more than four standard errors, and this is a much greater difference than is required to conclude that the means are significantly different. So this is not perfect either. With standard errors we could be sure than the means were not significantly different when the bars did overlap; and with confidence intervals we can be sure that the means are significantly different when the bars do not overlap – but the alternative cases are not clear cut for either type of bar. Can we somehow get the best of both worlds, so that the means are significantly different when the bars do not overlap, and the means are not significantly different when the bars do overlap?

The answer is yes, we can, if we use LSD bars (LSD stands for 'least significant difference'). Let's revisit the formula for Student's t-test:

$$t = \frac{\text{a difference}}{\text{standard error of the difference}}$$

and we say that the difference is significant when $t > 2$ (by the rule of thumb, or $t >$ qt(0.975,df) if we want to be more precise). We can rearrange this formula to find the smallest difference that we would regard as being significant. We can call this the least significant difference:

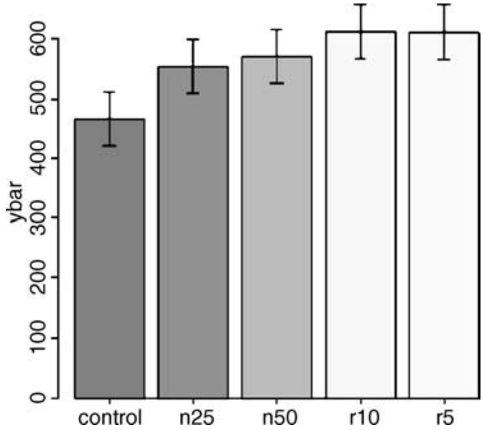
$$\text{LSD} = \mathsf{qt(0.975, df)} \times \text{standard error of a difference} \approx 2 \times \text{s.e.}_{\text{difference}}.$$

In our present example this is

```
qt(0.975,10)*sqrt(2*4961/6)
[ 1] 90.60794
```

because a difference is based on $12 - 2 = 10$ degrees of freedom. What we are saying is the two means would be significantly different if they differed by 90.61 or more. How can we show this graphically? We want overlapping bars to indicate a difference less than 90.61, and non-overlapping bars to represent a difference greater than 90.61. With a bit of thought you will realize that we need to draw bars that are LSD/2 in length, up and down from each mean. Let's try it with our current example:

```
lsd<-qt(0.975,10)*sqrt(2*4961/6)
lsdbars<-rep(lsd,5)/2
error.bars(ybar,lsdbars,labels)
```



Now we can interpret the significant differences visually. The control biomass is significantly lower than any of the four treatments, but none of the four treatments is significantly different from any other. The statistical analysis of this contrast is explained in detail in Chapter 12. Sadly, most journal editors insist on error bars of 1 s.e.. It is true that there are complicating issues to do with LSD bars (not least the vexed question of multiple comparisons; see p. 226), but at least LSD/2 bars do what was intended by the error plot (i.e. overlapping bars means non-significance and non-overlapping bars means significance); neither standard errors nor confidence intervals can say that. A better option might be to use box and whisker plots with the notch = T option to indicate significance (see p. 77).

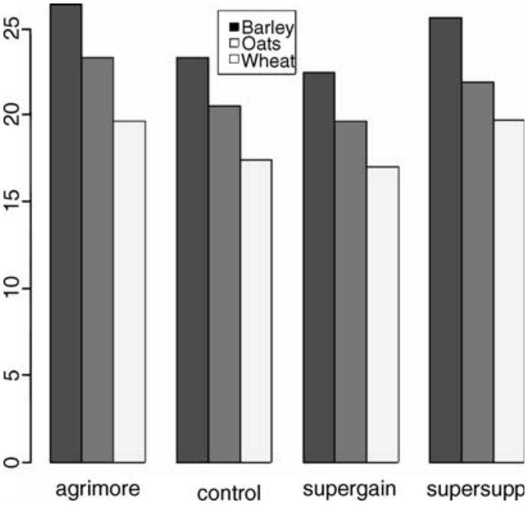
Factorial Experiments

A factorial experiment has two or more factors, each with two or more levels, plus replication for each combination of factor levels. This means that we can investigate statistical interactions, in which the response to one factor depends on the level of another factor. Our example comes from a farm-scale trial of animal diets. There are two factors: diet and supplement. Diet is a factor with three levels: barley, oats and wheat. Supplement is a factor with four levels: agrimore, control, supergain and supersupp. The response variable is weight gain after 6 weeks.

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

Data inspection is carried out using barplot (note the use of beside = T to get the bars in adjacent clusters rather than vertical stacks):

```
barplot(tapply(gain,list(diet,supplement),mean),beside = T)
```



Note that the second factor in the list (supplement) appears as groups of bars from left to right in alphabetical order by factor level, from ‘agrimore’ to ‘supersupp’. The second factor (diet) appears as three levels within each group of bars: on your screen red = barley, orange = oats, yellow = wheat, again in alphabetical order by factor level. We should really add a key to explain the levels of diet (I used locator(1) to find the appropriate coordinates for the legend at (6.3,26); this is the **top left** corner of the box):

```
labs < -c("Barley","Oats","Wheat")
cols < -c("red","orange","yellow")
legend(6.3,26,labs,fill = cols)
```

We inspect the mean values using tapply as usual:

```
tapply(gain,list(diet,supplement),mean)
```

```
        agrimore   control  supergain   supersupp
barley  26.34848   23.29665  22.46612   25.57530
oats    23.29838   20.49366  19.66300   21.86023
wheat   19.63907   17.40552  17.01243   19.66834
```

Now we use aov or lm to fit a factorial Anova (the choice affects whether we get an Anova table or a list of parameter estimates as the default output from summary). We estimate parameters for the main effects of each level of diet and each level of supplement, plus terms for the interaction between diet and supplement. Interaction degrees of freedom are the product of the degrees of freedom of the component terms, i.e. $(3 - 1) \times (4 - 1) = 6$. The model is gain ~ diet + supplement + diet:supplement, but this can be simplified using the asterisk notation like this:

```
model<-aov(gain~diet*supplement)
summary(model)
```

```
                Df   Sum Sq   Mean Sq  F value      Pr(>F)
diet             2  287.171   143.586  83.5201  2.998e-14  ***
supplement       3   91.881    30.627  17.8150  2.952e-07  ***
diet: supplement 6    3.406     0.568   0.3302     0.9166
Residuals       36   61.890     1.719
```

The Anova table shows that there is no hint of any interaction between the two explanatory variables ($p = 0.9166$); evidently the effects of diet and supplement are additive. The disadvantage of the Anova table is that it does not show us the effect sizes, and does not allow us to work out how many levels of each of the two factors are significantly different. As a preliminary to model simplification, summary.lm is often more useful than summary.aov:

```
summary.lm(model)
```

```
Coefficients:
                               Estimate  Std. Error  t value   Pr(>|t|)
(Intercept)                     26.3485      0.6556   40.191    < 2e-16  ***
dietoats                        -3.0501      0.9271   -3.290   0.002248   **
dietwheat                       -6.7094      0.9271   -7.237   1.61e-08  ***
supplementcontrol               -3.0518      0.9271   -3.292   0.002237   **
supplementsupergain             -3.8824      0.9271   -4.187   0.000174  ***
supplementsupersupp             -0.7732      0.9271   -0.834   0.409816
dietoats:supplementcontrol       0.2471      1.3112    0.188   0.851571
dietwheat:supplementcontrol      0.8183      1.3112    0.624   0.536512
dietoats:supplementsupergain     0.2470      1.3112    0.188   0.851652
dietwheat:supplementsupergain    1.2557      1.3112    0.958   0.344601
dietoats:supplementsupersupp    -0.6650      1.3112   -0.507   0.615135
```

```
dietwheat:supplementsupersupp        0.8024     1.3112     0.612      0.544381

Residual standard error: 1.311 on 36 degrees of freedom
Multiple R-Squared: 0.8607,  Adjusted R-squared: 0.8182
F-statistic: 20.22 on 11 and 36 DF,  p-value: 3.295e-012
```

This is a rather complex model, because there are 12 estimated parameters (the number of rows in the table), six main effects and six interactions. The output re-emphasizes that none of the interaction terms is significant, but it suggests that the minimal adequate model will require five parameters: an intercept, a difference due to oats, a difference due to wheat, a difference due to control and a difference due to supergain (these are the five rows with significance stars). This draws attention to the main shortcoming of using treatment contrasts as the default. If you look carefully at the table, you will see that the effect sizes of two of the supplements, control and supergain, are not significantly different from one another. You need lots of practice at doing *t*-tests in your head, to be able to do this quickly. Ignoring the signs (because the signs are negative for both of them) we have 3.05 vs. 3.99, a difference of 0.94. But look at the associated standard errors (both 0.927); the difference is only about 1 s.e. of a difference between two means. For significance, we would need roughly 2 s.e.'s (remember the rule of thumb, in which $t \geq 2$ is significant; see p. 68). The rows get starred in the significance column because treatments contrasts compare all the main effects in the rows with the intercept (where each factor is set to its first level in the alphabet, namely agrimore and barley in this case). When, as here, several factor levels are different from the intercept, but not different from one another, they all get significance stars. This means that you cannot count up the number of rows with stars in order to determine the number of significantly different factor levels. We first simplify the model by leaving out the interaction terms:

```
model<-aov(gain~diet+supplement)
summary.lm(model)
```

```
Coefficients:
                      Estimate   Std. Error   t value    Pr(>|t|)
(Intercept)            26.1230       0.4408    59.258      <2e-16  ***
dietoats               -3.0928       0.4408    -7.016    1.38e-08  ***
dietwheat              -5.9903       0.4408   -13.589      <2e-16  ***
supplementcontrol      -2.6967       0.5090    -5.298    4.03e-06  ***
supplementsupergain    -3.3815       0.5090    -6.643    4.72e-08  ***
supplementsupersupp    -0.7274       0.5090    -1.429       0.160
```

It is clear that we need to retain all three levels of diet (oats differ from wheat by 5.99 – 3.10 = 2.89 with a standard error of 0.44). It is not clear that we need four levels of supplement, however. Supersupp is not obviously different from the agrimore (0.727 with s.e. = 0.509). Nor is supergain obviously different from the un-supplemented control animals (3.38 – 2.70 = 0.68). We shall try a new two-level factor to replace the four-level supplement, and see if this significantly reduces the model's explanatory power. Agrimore and supersupp are re-coded as 'best' and control and supergain as 'worst':

```
supp2 < -factor(supplement)
levels(supp2)
```

```
[ 1] "agrimore"  "control"  "supergain"  "supersupp"
```

```
levels(supp2)[c(1,4)] < -"best"
levels(supp2)[c(2,3)] < -"worst"
levels(supp2)
```

```
[ 1] "best"  "worst"
```

Now we can compare the two models

```
model2 < -aov(gain ~ diet + supp2)
anova(model,model2)
```

```
Analysis of Variance Table

Model 1: gain ~ diet + supplement
Model 2: gain ~ diet + supp2

Res.Df   RSS Df  Sum of Sq      F Pr(>F)
1   42   65.296
2   44   71.284 -2 -5.988  1.9257    0.1584
```

The simpler model two has saved two degrees and is not significantly worse than the more complex model ($p = 0.158$). This is the minimal adequate model – all of the parameters are significantly different from zero and from one another:

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

```
Coefficients:
              Estimate  Std. Error   t value   Pr(>|t|)
(Intercept)    25.7593      0.3674    70.106     <2e-16   ***
dietoats       -3.0928      0.4500    -6.873   1.76e-08   ***
dietwheat      -5.9903      0.4500   -13.311     <2e-16   ***
supp2worst     -2.6754      0.3674    -7.281   4.43e-09   ***

Residual standard error: 1.273 on 44 degrees of freedom
Multiple R-Squared: 0.8396,     Adjusted R-squared: 0.8286
F-statistic: 76.76 on 3 and 44 DF, p-value:          0
```

Model simplification has reduced our initial 12-parameter model to a four-parameter model.

Pseudoreplication: Nested Designs and Split Plots

The model-fitting functions aov and lme have the facility to deal with complicated error structures. Detailed analysis of these topics is beyond the scope of this book (see

Statistical Computing, Crawley 2002, for worked examples), but it is important that you can recognize them, and hence avoid the pitfalls of pseudoreplication. There are two general cases:

- nested sampling, as when repeated measurements are taken from the same individual, or observational studies are conduced at several different spatial scales (mostly random effects), and
- split-plot analysis, as when designed experiments have different treatments applied to plots of different sizes (mostly fixed effects)

Split-plot Experiments

In a split-plot experiment, different treatments are applied to plots of different sizes. Each different plot size is associated with its own error variance, so instead of having one error variance (as in all the Anova tables up to this point), we have as many error terms as there are different plot sizes. The analysis is presented as a series of component Anova tables, one for each plot size, in a hierarchy from the largest plot size with the lowest replication at the top, down to the smallest plot size with the greatest replication at the bottom.

The example refers to a designed field experiment on crop yield with three treatments: irrigation (with two levels, irrigated or not), sowing density (with three levels, low, medium and high), and fertilizer application (with three levels, low, medium and high).

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

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

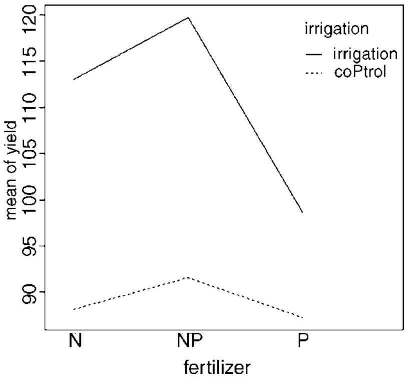
The largest plots were the four whole fields (block), each of which was split in half, and irrigation was allocated at random to one half of the field. Each irrigation plot was split into three, and one of three different seed-sowing densities (low, medium or high) was allocated at random (independently for each level of irrigation and each block). Finally, each density plot was divided into three and one of three fertilizer nutrient treatments (N, P, or N and P together) was allocated at random. The model formula is specified as a factorial, using the asterisk notation. The error structure is defined in the Error() term, with the plot sizes listed from left to right, from largest to smallest, with each variable separated by the slash operator /. Note that the smallest plot size, fertilizer, does not need to appear in the error term:

```
model <-aov(yield~irrigation*density*fertilizer+Error(block/irrigation/density))
summary(model)
```

```
Error: block
                     Df   Sum Sq   Mean Sq  F value       Pr(>F)
Residuals             3  194.444    64.815

Error: block:irrigation
                     Df   Sum Sq   Mean Sq  F value       Pr(>F)
irrigation            1   8277.6    8277.6   17.590     0.02473    *
Residuals             3   1411.8     470.6

Error: block:irrigation:density
                     Df   Sum Sq   Mean Sq  F value       Pr(>F)
density               2  1758.36    879.18   3.7842    0.05318.
irrigation: density   2  2747.03   1373.51   5.9119     0.01633    *
Residuals            12  2787.94    232.33

Error: Within
                     Df   Sum Sq   Mean Sq  F value       Pr(>F)
fertilizer            2  1977.44    988.72  11.4493   0.0001418  ***
irrigation: fertilizer 2  953.44    476.72   5.5204   0.0081078   **
density: fertilizer   4   304.89     76.22   0.8826   0.4840526
irrigation:           4   234.72     58.68   0.6795   0.6106672
  density: fertilizer
Residuals            36  3108.83     86.36
```

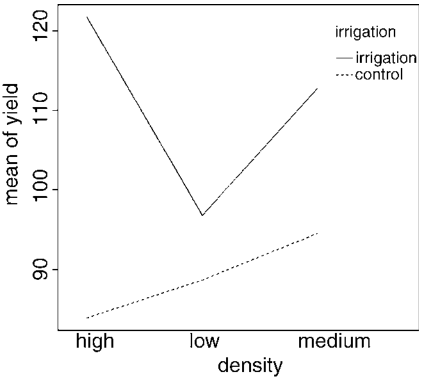
Here, you see the four Anova tables, one for each plot size: blocks are the biggest plots, half blocks get the irrigation treatment, one third of each half block gets a sowing density treatment, and one third of a sowing density treatment gets each fertilizer treatment. Note that the non-significant main effect for density ($p = 0.053$) does **not** mean that density is unimportant, because density appears in a significant interaction with irrigation (the density terms cancel out, when averaged over the two irrigation treatments; see below). The best way to understand the two significant interaction terms is to plot them using interaction.plot like this

```
interaction.plot(fertilizer,irrigation,yield)
```



Irrigation increases yield proportionately more on the N-fertilized plots than on the P-fertilized plots. The irrigation/density interaction is more complicated:

```
interaction.plot(density,irrigation,yield)
```



On the irrigated plots, yield is minimal on the low-density plots, but on control plots yield is minimal on the high-density plots.

Random Effects and Nested Designs

Mixed effects models are so called because the explanatory variables are a mixture of fixed effects and random effects:

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

A random effect should be thought of as coming from a population of effects: the existence of this population is an extra assumption. We speak of **prediction of random effects**, rather than estimation; we **estimate** fixed effects from data, but we intend to make predictions about the population from which our random effects were sampled. Fixed effects are unknown constants to be estimated from the data. Random effects govern the variance–covariance structure of the response variable. The fixed effects are often experimental treatments that were applied under our direction, and the random effects are either categorical or continuous variables that are distinguished by the fact that we are typically not interested in the parameter values, but only in the variance they explain.

One or more of the explanatory variables represents **grouping** in time or in space. 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**. Mixed effects models take care of this non-independence of errors by modelling the covariance structure introduced by the grouping of the data. A major benefit of random effects models is that they economize on the number of degrees of freedom used up by the factor levels. Instead of estimating a mean for every single factor level, the random effects model estimates the distribution of the means (usually as the standard deviation of the differences of the factor-level means around an overall mean). Mixed effects models are particularly useful in cases where there is temporal pseudoreplication (repeated measurements) and/or spatial pseudoreplication (e.g. nested designs or split-plot experiments). These models can allow for:

- spatial autocorrelation between neighbours,
- temporal autocorrelation across repeated measures on the same individuals,
- differences in the mean response between blocks in a field experiment, and
- differences between subjects in a medical trial involving repeated measures.

The point is that we really do not want to waste precious degrees of freedom in estimating parameters for each of the separate levels of the categorical random variables. On the other hand, we do want to make use of the all measurements we have taken, but because of the pseudoreplication we want to take account of both the

- correlation structure, used to model within-group correlation associated with temporal and spatial dependencies, using **correlation**, and
- variance function, used to model non-constant variance in the within-group errors using **weights**.

Fixed or Random Effects?

It is difficult without lots of experience to know when to use categorical explanatory variables as fixed effects and when as random effects. Some guidelines are given below.

- Am I interested in the effect sizes ? Yes, means fixed effects.
- Is it reasonable to suppose that the factor levels come from a population of levels? Yes, means random effects.
- Are there enough levels of the factor in the data from on which to base an estimate of the variance of the population of effects? No, means fixed effects.
- Are the factor levels informative? Yes, means fixed effects.
- Are the factor levels just numeric labels ? Yes, means random effects.
- Am I mostly interested in making inferences about the distribution of effects, based on the random sample of effects represented in the dataframe? Yes, means random effects.
- Is there hierarchical structure? Yes, means you need to ask whether the data are experimental or observations.

- Is it an hierarchical experiment, where the factor levels are experimental manipulations? Yes, means fixed effects in a split-plot design (see p. 176).
- Is it an hierarchical observational study? Yes, means random effects, perhaps in a variance components analysis (see p. 181).
- When your model contains both fixed and random effects, use mixed effects models.
- If your model structure is linear, use linear mixed effects, **lme**.
- Otherwise, specify the model equation and use non-linear mixed effects, **nlme**.

Removing the Pseudoreplication

The extreme response to pseudoreplication in a data set is simply to eliminate it. Spatial pseudoreplication can be averaged away and temporal pseudoreplication can be dealt with by carrying out separate Anovas, one at each time. This approach has two major weaknesses:

- it cannot address questions about treatment effects that relate to the longitudinal development of the mean response profiles (e.g. differences in growth rates between successive times);
- inferences made with each of the separate analyses are not independent, and it is not always clear how they should be combined.

Analysis of Longitudinal Data

The key feature of longitudinal data is that the same individuals are measured repeatedly through time. This would represent temporal pseudoreplication if the data were used uncritically in regression or Anova. The set of observations on one individual subject will tend to be positively correlated and this correlation needs to be taken into account in carrying out the analysis. The alternative is a cross-sectional study, with all the data gathered at a single point in time, in which each individual contributes a single data point. The advantage of longitudinal studies is that they are capable of separating **age effects** from **cohort effects**; these are inextricably confounded in cross-sectional studies. This is particularly important when differences between years mean that cohorts originating at different times experience different conditions, so that individuals of the same age in different cohorts would be expected to differ. There are two extreme cases in longitudinal studies:

- a few measurements on a large number of individuals,
- a large number of measurements on a few individuals,

In the first case it is difficult to fit an accurate model for change within individuals, but treatment effects are likely to be tested effectively. In the second case, it is possible to get an accurate model of the way that individuals change though time, but there is less power for testing the significance of treatment effects, especially if variation from individual to individual is large. In the first case, less attention will be paid to estimating the correlation structure, while in the second case the covariance model will be the principal focus of attention. The aims are:

- to estimate the average time course of a process,
- to characterize the degree of heterogeneity from individual to individual in the rate of the process,
- to identify the factors associated with both of these, including possible cohort effects.

The response is not the individual measurement, but the **sequence of measurements** on an individual subject. This enables us to distinguish between age effects and year effects (see Diggle *et al.* (1994) for details).

Derived Variable Analysis

The idea here is to get rid of the pseudoreplication by reducing the repeated measures into a set of summary statistics (slopes, intercepts or means), then **analyse these summary statistics** using standard parametric techniques like Anova or regression. The technique is weak when the values of the explanatory variables change through time. Derived variable analysis makes most sense when it is based on the parameters of scientifically interpretable non-linear models from each time sequence. However, the best model from a theoretical perspective may not be the best model from the statistical point of view.

There are three qualitatively different sources of random variation:

- **random effects**: experimental units differ (e.g. genotype, history, size, physiological condition) so that there are intrinsically high responders and other low responders,
- **serial correlation**: there may be time-varying stochastic variation within a unit (e.g. market forces, physiology, ecological succession, immunity) so that correlation depends on the time separation of pairs of measurements on the same individual, with correlation weakening with the passage of time,
- **measurement error**: the assay technique may introduce an element of correlation (e.g. shared bioassay of closely spaced samples; different assay of later specimens).

Variance Components Analysis (VCA)

For random effects we are often more interested in the question of how much of the variation in the response variable can be attributed to a given factor, than we are in estimating means or assessing the significance of differences between means. This procedure is called variance components analysis.

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

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

This classic example of pseudoreplication comes from Snedecor and Cochran's *Statistical Methods* (1980). Three experimental treatments were administered to rats, and the glycogen contents of the rats' livers were analysed as the response variable. This was the set-up – there were two rats per treatment, so the total sample was $n = 3 \times 2 = 6$. The tricky bit was that after each rat was killed, its liver was cut up into three pieces: a left-hand bit, a central bit and a right-hand bit. So now there are six rats each producing three bits of liver, for a total of $6 \times 3 = 18$ numbers. Finally, two separate preparations were made from each macerated bit of liver, to assess the measurement error associated with the analytical machinery. At this point there are $2 \times 18 = 36$ numbers in the dataframe as a whole. The factor levels are numbers, so we need to declare the explanatory variables to be categorical before we begin:

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

Here is the analysis done the **wrong** way:

```
model<-aov(Glycogen~Treatment)
summary(model)

             Df   Sum Sq   Mean Sq  F value       Pr(>F)
Treatment     2  1557.56    778.78   14.498  3.031e-05***
Residuals    33  1772.67     53.72
```

Treatment has a highly significant effect on liver glycogen content ($p = 0.00003$). This is wrong! We have committed a classic error of pseudoreplication. Look at the error line in the Anova table: it says the residuals have 33 degrees of freedom. However, there were only six rats in the whole experiment, so the error d.f. has to be $6 - 1 - 2 = 3$ (not 33)! Here is the analysis of variance done properly, averaging away the pseudoreplication:

```
tt<-as.numeric(Treatment)
yv<-tapply(Glycogen,list(Treatment,Rat),mean)
tv<-tapply(tt,list(Treatment,Rat),mean)

model<-aov(as.vector(yv)~factor(as.vector(tv)))
summary(model)

                       Df   Sum Sq  Mean Sq F value  Pr(>F)
factor(as.vector(tv))   2  259.593  129.796   2.929  0.1971
Residuals               3  132.944   44.315
```

Now the error degrees of freedom are correct (d.f. $= 3$, not 33), and the interpretation is completely different: there are no significant differences in liver glycogen under the three experimental treatments ($p = 0.1971$).

There are two different ways of doing the analysis properly in R: Anova with multiple error terms (aov) or linear mixed effects models (lme). The problem is that the bits of the same liver are pseudoreplicates because they are spatially correlated (they come from the same rat); they are not independent, as required if they are to be true replicates. Likewise, the two preparations from each liver bit are very highly correlated (the livers were macerated before the preparations were taken, so they are essentially the same sample (certainly not independent replicates of the experimental treatments).

Here is the correct analysis using aov with multiple error terms. In the error term we start with the largest scale (treatment), then rats within treatments, then liver bits within rats within treatments. Finally, there were replicated measurements (two preparations) made for each bit of liver.

```
model2 < -aov(Glycogen ~ Treatment + Error(Treatment/Rat/Liver))
summary(model2)
```

```
Error: Treatment
             Df    Sum Sq    Mean Sq
Treatment     2   1557.56     778.78

Error: Treatment:Rat
             Df    Sum Sq    Mean Sq   F value     Pr(>F)
Residuals     3    797.67     265.89

Error: Treatment:Rat:Liver
             Df    Sum Sq    Mean Sq   F value     Pr(>F)
Residuals    12     594.0       49.5

Error: Within
             Df    Sum Sq    Mean Sq   F value     Pr(>F)
Residuals    18    381.00      21.17
```

You can do the correct, non-pseudoreplicated analysis of variance from this output (Box 9.2).

Box 9.2. Sums of squares in hierarchical designs

The trick to understanding these sums of squares is to appreciate that with nested categorical explanatory variables (random effects) the correction factor, which is subtracted from the sum of squared subtotals, is **not** the conventional $(\sum y)^2/kn$. Instead, the correction factor is the uncorrected sum of squared subtotals from the level in the hierarchy immediately above the level in question. This is very hard to see without lots of practice. The total sum of squares, *SSY*, and the treatment sum of squares, *SSA*, are computed in the usual way (see Box 9.1):

$$SSY = \sum y^2 - \frac{(\sum y)^2}{n}$$
$$SSA = \frac{\sum_{i=1}^{k} C_i^2}{n} - \frac{(\sum y)^2}{kn}.$$

The analysis is easiest to understand in the context of an example. For the rats data, the treatment totals were based on 12 numbers (two rats, three liver bits per rat and two preparations per liver bit). In this case, in the formula for *SSA* above, $n = 12$ and $kn = 36$. We need to calculate sums of squares for rats within treatments, SS_{Rats}, liver bits within rats within treatments, $SS_{\text{Liver bits}}$, and preparations within liver bits within rats within treatments, $SS_{\text{Preparations}}$:

$$SS_{\text{Rats}} = \frac{\sum R^2}{6} - \frac{\sum C^2}{12}$$
$$SS_{\text{Liverbits}} = \frac{\sum L^2}{2} - \frac{\sum R^2}{6}$$
$$SS_{\text{Preparations}} = \frac{\sum y^2}{1} - \frac{\sum R^2}{6}.$$

The correction factor at any level is **the uncorrected sum of squares from the level above**. The last sum of squares could have been computed by difference:

$$SS_{\text{Preparations}} = SSY - SSA - SS_{\text{Rats}} - SS_{\text{Liverbits}}.$$

The *F* test for equality of the treatment means is the treatment variance divided by the 'rats within treatment variance' from the row immediately beneath: $F = 778.78/265.89 = 2.928956$, with 2 d.f. in the numerator and 3 d.f. in the denominator (as we obtained in the correct Anova, above).

To turn this into a variance components analysis we need to do a little work. The mean squares are converted into variance components like this:

$$\text{Residuals} = \text{preparations within liver bits}: \ \text{unchanged} = 21.17$$
$$\text{Liver bits within rats within treatments}: (49.5 - 21.17)/2 = 14.165$$
$$\text{Rats within treatments}: (265.89 - 49.5)/6 = 36.065$$

You divide the difference in variance by the number of numbers in the level below (i.e. two preparations per liver bit, and six preparations per rat, in this case).

What is the Difference Between Split-plot and Hierarchical Samples?

Split-plot experiments have informative factor levels. Hierarchical samples have uninformative factor levels. That's the distinction. In the irrigation experiment, the factor levels were as follows:

```
levels(density)
```

```
[ 1] "high" "low" "medium"
```

```
levels(fertilizer)
```

```
[ 1] "N" "NP" "P"
```

They show the density of seed sown, and the kind of fertilizer applied – they are informative. Here are the factor levels from the rats experiment:

```
levels(Rat)
```

```
[ 1] "1" "2"
```

```
levels(Liver)
```

```
[ 1] "1"  "2"  "3"
```

These factor levels are uninformative, because rat number 2 in treatment 1 has nothing in common with rat number 2 in treatment 2, or with rat number 2 in treatment 3. Liver bit number 3 from rat 1 has nothing in common with liver bit number 3 from rat 2. Note, however, that numbered factor levels are **not** always uninformative: treatment levels 1, 2 and 3 are informative: 1 is the control, 2 is a diet supplement and 3 is a combination of two supplements.

When the factor levels are informative, the variable is known as a **fixed effect**. When the factor levels are uninformative, the variable is known as a **random effect**. Generally, we are interested in fixed effects as they influence the mean, and in random effects as they influence the variance. We tend not to speak of effect-sizes attributable to random effects, but effect-sizes and their standard errors are often the principal focus when we have fixed effects. Thus, irrigation, density and fertilizer are fixed effects, and rat and liver-bit are random effects.