

## Blocking and repeated measures in ANOVA:

The idea here is that we have some effect we want to “eliminate”, and some effect that we're interested in.

*Randomized complete block:*

In many ways this resembles a two way mixed model ANOVA. But instead of being interested in the variation (the random variation), we're now trying to get rid of it.

Let's take a look at an example:

We have rats from four suppliers. We want to evaluate the effect of a new diabetes drug that increases insulin production and compare this to an old drug at the same time. We're worried that since our rats are from three different suppliers we may get a “rat” effect which might confuse the results we're interested in.

We decide that we will take three rats from each supplier and randomly assign it to one of the following treatments:

	Rat 1	Rat 2	Rat 3
supp 1	control (a)	new drug (b)	old drug (c)
supp 2	new drug (d)	control (e)	old drug (f)
supp 3	old drug (g)	new drug (h)	control (i)
supp 4	old drug (j)	control (k)	new drug (l)

where the lower case letters are the measured insulin production.

Now when we re-arrange our data, we get the following:

	Control	Old drug	New drug
supp 1	a	c	b
supp 2	e	f	d
supp 3	i	g	h
supp 4	k	j	l

Notice that this now looks a little bit like a two way ANOVA with “drug” as one factor and “supplier” as the other factor.

This way, we can eliminate the variation due to supplier.

Also note that there is only one observation at each “crossing” of drug and supplier.

This is very similar to example 12.4 on page 271.

This is called a randomized block design.

Subjects are assigned at random within each block. In this case the “block” is the factor we're not really interested in - “supplier”.

So how is this analyzed?

Most of the equations stay the same, but there are some important points:

It is impossible to calculate the interaction because there are no replicates within each combination of “factors”.

There is no  $SS_{\text{within}}$ , and to test for interaction we need to do  $SS_{\text{interaction}}/SS_{\text{within}}$

Usually, we only have one observation for every combination of “factors”.

(You'd need at least two observations to calculate a  $SS_{\text{within}}$ ).

However, there is still “variation” that's unaccounted for.

We have variation due to supplier, and variation due to drug, but these two sources of variation do not add up to the “total” variation that's present.

Very similar to  $SS_{\text{within}}$ , we now have  $SS_{\text{error}} = SS_{\text{remainder}} = SS_{\text{residual}}$

(three different names for the same thing - notice that  $SS_{\text{within}}$  is actually a special form of  $SS_{\text{residuals}}$ , so we've been using this the whole time:

R even calls this  $SS_{\text{residuals}}$  in any ANOVA printout.

So basically, it's similar to  $SS_{\text{within}}$ , but calculated differently.

For most purposes, it's easiest to calculate as:

$$SS_{\text{total}} - SS_{\text{factor A}} - SS_{\text{factor B}} = SS_{\text{residual}}$$

The precise calculations are very similar to what we've done so far:

$$SS_{\text{total}} = \text{the total sum of squares} = \text{sum of (each obs. - grand mean)}^2$$

(see 12.28, p. 267 for formula)

$$SS_{\text{factor A}} = \text{sum of squares for between groups for factor A}$$

$$\text{sum of } b \times (\text{mean for each column of factor a} - \text{grand mean})^2$$

(see 12.30, p. 268 for formula)

$$SS_{\text{factor B}} = \text{sum of squares for between groups for factor B}$$

$$\text{sum of } a \times (\text{mean for each row of factor b} - \text{grand mean})^2$$

(see 12.31, p. 268 for formula)

And  $SS_{\text{residual}}$  as above

**Comment:** the formula references in table 12.4 on p. 268 are wrong. Increase each reference by 1 (e.g., 12.27 should be 12.28)

Basically, it's very similar to what we did with two way ANOVA's, except that there is no interaction (and, incidentally,  $SS_{\text{cells}} = SS_{\text{total}}$ )

We arrange everything into an ANOVA table:

Source	SS	df	MS	F
factor a	$SS_{\text{factor a}}$	a-1	$SS_{\text{factor a}}/(a - 1)$	$MS_{\text{factor a}}/MS_{\text{residual}}$
factor b	$SS_{\text{factor b}}$	b-1	$SS_{\text{factor b}}/(b - 1)$	$MS_{\text{factor b}}/MS_{\text{residual}}$ ***
residual	$SS_{\text{residual}}$	(a-1)(b-1)	$SS_{\text{residual}}/(a-1)(b-1)$	
TOTAL	$SS_{\text{total}}$	N-1		

\*\*\* often we're not interested in testing this since this is a “confounding” factor. It just gets in the way, and we want to “remove” the variation caused by this from our analysis.

Also notice how we calculate F; we compare all the MS's with the  $MS_{\text{residual}}$  (a lot less confusing that the mixed model stuff we were discussing).

Some texts will refer to this as a one-way design since we're really only interested in the effects of one of the factors.

So let's do an example (12.4, p. 271):

The setup is very similar to what was described above with the mice. But this time we're investigating the effects of anesthetic on cats:

We get 15 cats, but each group of 5 cats comes from a different supplier.

Each supplier may feed the cats different things, or keep them housed differently.

This might cause cats from different suppliers to react differently.

We want to test the effects of three different anesthetics:

Go through Example 12.4.

Note how we take each cat from each supplier and randomly assign it to one of the treatments:

We measure the amount of time it takes for the anesthetic to work.

We then arrange these into blocks vs. treatments, as on the bottom of the page.

We calculate the various SS's, as described above, and wind up with the ANOVA table at the top of page 272.

From this we calculate F, and find that the anesthetics are not all equally effective.

Here's how to do the same example in R:

```
time <- scan()  
8.25 11.25 10.75 11.00 12.50 11.75 10.25 12.00 11.25 9.50 9.75  
9.00 8.75 11.00 10.00  
  
treat <- scan()  
1 2 3 1 2 3 1 2 3 1 2 3 1 2 3  
  
supp <- scan()  
1 1 1 2 2 2 3 3 3 4 4 4 5 5 5
```

(notice that you can use numbers for treatment effects or blocking effects, you just need to tell R that they're factors:)

```
treat <- factor(treat)  
supp <- factor(supp)
```

and then finally, we can do the ANOVA:

```
cats <- aov(time ~ treat + supp)  
summary(cats)
```

Notice the “+” this time. This means to do the ANOVA *without* interaction.

If you try to get an interaction (i.e., do “treat \* supp” in the aov statement), R won't give you an error. It simply won't print any F tests!

And we get the R printout:

```
              Df Sum Sq Mean Sq F value    Pr(>F)    **  
treat          2  7.708   3.854   10.423 0.00592    **  
supp           4 11.067   2.767    7.482 0.00823    **  
Residuals     8  2.958   0.370  
---  
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Which is identical to what is given at the top of page 272.

If you wanted to investigate the effects of the anesthetics in more detail, you could follow up with Tukey's:

```
TukeyHSD(cats)
```

And get the following results:



*Repeated measures:*

A repeated measures design is almost identical to the randomized complete block design we just discussed.

Usually, though, we test the same subject on all three treatments. It's a bit like a "paired" design, with more than two samples.

Suppose we want to determine the effect of our anesthetic.

We take each cat, administer anesthetic a, and take our measurement

Let each cat recover. Administer anesthetic b, take our measurement

Etc.

The analysis would be identical, but this time "cat" would be the blocking factor rather than the supplier.

You should still select your cats at random.

The order in which you administer the anesthetic should be random (if possible) as well.

That's not always possible. Suppose you select 24 people and ask them to do strength training.

You're interested in the effect of strength training after 20 days and after 40 days.

Obviously you can't do this in a random order!

Let's do an example of a repeated measures design:

Here's an example (loosely based on another text):

We want to investigate the effect of strength training on 7 volunteers. We get the following results (ability to lift a certain weight).

subject	before	1 month	2 months
1	100	115	125
2	110	125	135
3	90	105	120
4	110	130	140
5	125	140	150
6	130	140	155
7	105	125	140

First, let's set up our hypothesis:

$H_0: \mu_1 = \mu_2 = \mu_3$   
pick  $\alpha = 0.05$

$H_1$ : at least one mean is different

Now let's calculate the various SS's (I'm not doing this by hand!):

```
SSsubject = 2995.24
SSmonth = 2730.95
SSresidual = 69.05
```

And arrange everything into an ANOVA table (here's the R printout):

```
              Df  Sum Sq Mean Sq F value    Pr(>F)
subject      6 2995.24  499.21  86.759 3.525e-09 ***
month        2 2730.95 1365.48 237.310 2.249e-10 ***
Residuals   12   69.05    5.75
```

And we notice that strength training does have an effect. We follow up with Tukey's to get the following:

```
TukeyHSD(training)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = strength ~ month + subj)

$month
      diff      lwr      upr    p adj
1-0 15.71429 12.29360 19.13497 1.0e-07
2-0 27.85714 24.43646 31.27783 0.0e+00
2-1 12.14286  8.72217 15.56354 1.8e-06
```

The subject comparisons are not shown since they're not interesting.

Incidentally, what would happen if we did this incorrectly?

In other words, what would happen if we blindly used a one way ANOVA on these data, without accounting for the fact that it's the same subject for all three observations of the treatment?

Here's the ANOVA table:

```
              Df Sum Sq Mean Sq F value    Pr(>F)
month        2   2731  1365.5    8.021 0.00323 **
Residuals   18   3064   170.2
```

What happened? Notice that the p-value went way up (it's much higher). In other words, the repeated measures design is much more powerful (it has a much smaller p-value).

In fact, notice Tukey's:

```
Tukey multiple comparisons of means
  95% family-wise confidence level

Fit: aov(formula = strength ~ month)
```

\$month		diff	lwr	upr	p adj
1-0	15.71429	-2.085009	33.51358	0.0890247	
2-0	27.85714	10.057848	45.65644	0.0023317	
2-1	12.14286	-5.656437	29.94215	0.2176453	

Instead of showing us that all three months are different, it can only find a difference between month 0 and month 2.

Always use the correct design/analysis. It generally gives you much better results.

*We're still not done with ANOVA. We still want to look at the following designs:*

Nested

Multivariate = MANOVA (only briefly)

And we want to look at non-parametric equivalents (Kruskal-Wallis).