Lecture 32

Random Effects, Nested Effects, and Experimental Design

The analysis of designed experiments often involves some form of a General Linear Model analysis. The problem is that there are an infinite number of possible designs. A course covering them often will fail to cover one exactly like what you need for your experiment. There are many important components to an arbitrary experiment, many of which we have already discusses: predictors can be continuous or categorical. Categorical predictors are equivalent to including dichotomous indicator variables for the categories. Interactions are products of terms and mean that the responses are not in parallel to changes in the predictor variables, but depend on the combination of predictor variables. There are two other major components to the general theory of experimental design: random effects and nesting.

Random Effects

A fixed effect is a treatment/factor condition that you control or can measure again. For instance, giving a patient $\boldsymbol{\alpha}$ interferon versus $\boldsymbol{\gamma}$ interferon is a fixed effect. A random effect is a treatment/factor condition that you do not control and which can be viewed as a random sample from a larger population. For instance, the patient to whom you are giving the treatment may be viewed as a random patient from the larger pool of all possible Hepatitis B patients instead of a fixed effect. The distinction does not effect the way you set up the model, but it can effect the way you analyze the model. For the simple inclusion of only one random effect, the analysis doesn't change. So, in the example of 2-way ANOVA without replication, whether the patients should be considered random or fixed effects is a philosophical consideration only: no aspect of the analysis will change. To explore how the analysis changes, we need to consider two random effects AND their interaction term. So let's change the example to the following:

Suppose you are interested in some Drosophila measurement, like wing diameter. There are various strains/lines of Drosophila and many labs around the country which raise these strains. Suppose you measure the wing diameter on flies from 10 labs that house the same 5 lines. Then the design looks like:

	Line 1	Line 2	Line 3	Line 4	Line 5
Lab 1	data	data	data	data	data
Lab 2	data	data	data	data	data
⋮	⋮	⋮	⋮	⋮	⋮
Lab 10	data	data	data	data	data

With both lab and line considered random effects, rather than fixed effects, the ANOVA model is

still

$$\text{Wing Diameter}_{\boldsymbol{i,j,k}} = \boldsymbol{\mu} + \text{Lab}_{\boldsymbol{i}} + \text{Line}_{\boldsymbol{j}} + \text{Lab x Line}_{\boldsymbol{i,j}} + \boldsymbol{\epsilon_{i,j,k}}$$

However, the interpretation is different. The question is no longer whether the means differ between labs, but whether there is a positive (as opposed to zero) variance in the random lab effect. Similarly, the question is no longer whether the means differ between lines, but whether there is a positive variance in the random line effect. And a significant interaction term means that there is a positive variance in the random lab by line interaction term. The analysis proceeds from the highest interaction term towards the single terms and you look at the expected mean squared errors:

$$\text{EMS}_{\text{Lab x Line}} = \boldsymbol{n\sigma^2_{\text{interaction}}} + \boldsymbol{\sigma^2_{\text{error}}}$$

where $\boldsymbol{n}$ is the sample size in each cell. There are approximations/other formulas for unbalanced designs. The test for a significant interaction in this random effects model is the same as that in the fixed effects model:

$$\boldsymbol{F} = \frac{\text{EMS}_{\text{Lab x Line}}}{\text{EMS}_{\text{error}}}$$

However, when you evaluate the significance of the main effects, things change. Since you are asking whether their variance is zero or not, you consider

$$\text{EMS}_{\text{Lab}} = \boldsymbol{nJ\sigma^2_{\text{Lab}}} + \boldsymbol{n\sigma^2_{\text{interaction}}} + \boldsymbol{\sigma^2_{\text{error}}}$$

where $\boldsymbol{J}$ is the number of lines (in our example, $\boldsymbol{J = 5}$).

Here, to test whether the variance in Labs is significant or not, we consider

$$\boldsymbol{F} = \frac{\text{EMS}_{\text{Lab}}}{\text{EMS}_{\text{interaction}}}$$

Similarly, with lines, the model gives

$$\text{EMS}_{\text{Line}} = \boldsymbol{nI\sigma^2_{\text{Line}}} + \boldsymbol{n\sigma^2_{\text{interaction}}} + \boldsymbol{\sigma^2_{\text{error}}}$$

where $\boldsymbol{I}$ is the number of labs (in our example, $\boldsymbol{I = 10}$) and the test is

$$\boldsymbol{F} = \frac{\text{EMS}_{\text{Lines}}}{\text{EMS}_{\text{interaction}}}$$

All decent software will make the correct comparisons for you these days (it used to be you had to figure out the correct denominator by hand. This wasn't as trivial as it seems since sometimes it involves a linear combination of terms rather than a single term in the model.)

Mixed Effects Model

A mixed effects model is simply one that has some random effects in it and some fixed effects in it. In matrix format these can be specified as

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\boldsymbol{u} + \boldsymbol{\epsilon}$$

where $\mathbf{Y}$ is the vector of responses, $\mathbf{X}$ is the matrix of fixed predictors including a column of 1's for the intercept, $\boldsymbol{\beta}$ is the vector of fixed effects, $\mathbf{Z}$ is the matrix of random predictors (in the example above, there would be indicator variables for the lines, labs, and interactions), $\boldsymbol{u}$ is the vector of random effects, and $\boldsymbol{\epsilon}$ is the residual error.

Nested Effects

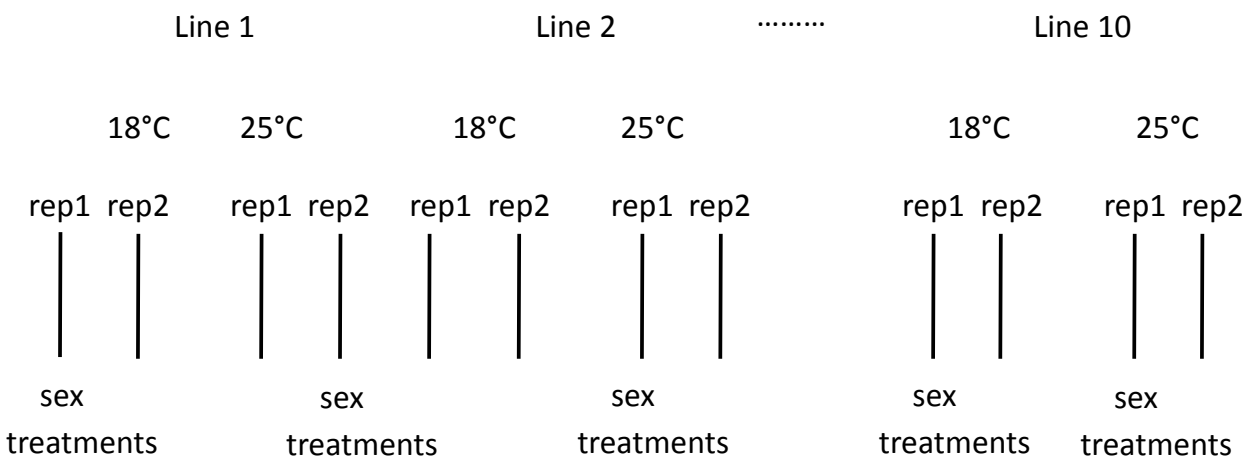
Sometimes a variable lives inside another. For example, suppose we did a field experiment with a fertilizer treatment in different plots. We are interested in the fertilizer treatment, but the plots might go into the ANOVA as a blocking factor since some plots are more favorable for plant growth than others. Now suppose, in addition, that we we have fields in very different environments (high altitude, medium altitude, and low altitude, for instance). The data might look like:

altitude	plot	treatment	growth
high	1	fertilizer	10.2
high	1	fertilizer	9
⋮	⋮	⋮	⋮
low	1	none	8

Plot 1 in high altitude has nothing to do with plot 1 in low altitude. They are both the "first" plots in each area, but they are not related. They are nested within the altitude. Nesting in Minitab is indicated with parentheses. Here it would be plot(altitude) to indicate that the plot variable is nested within the altitude variable.

An Example

Here is a more complicated example that I tried to help Suzanne Rutherford analyze. She had 10 Drosophila lines, 2 sexes, 3 treatments (2 distinct HSP-90 mutants and a control), two temperatures, and 2 replicates for each line and temperature. The proper picture is worth 1000 words (or more). Here is a "picture" of the design:



A full model will have line as random effect, sex, temperature, and treatment as fixed effects. Replicate is nested within line and temperature. Things nested within a random effect are random, so replicate is a random effect. Interactions between fixed effects and random effects are considered random. (Thus the state of being a random effect is dominant.) You cannot have an interaction between a nested variable and the thing it is nested inside. So the allowed 2-way interaction terms here are:

line × temperature
line × treatment
line × sex
treatment × temperature
sex × temperature
treatment × sex
treatment × replicate
sex × replicate

Exercises for Lecture 32

1. –

2. –