

## Model Based Statistics in Biology.

### Part IV. The General Linear Model. Multiple Explanatory Variables.

#### Chapter 13.7 Hierarchical ANOVA Random within Fixed To be completed

ReCap.	Part I (Chapters 1,2,3,4), Part II (Ch 5, 6, 7)	
ReCap	Part III (Ch 9, 10, 11)	
ReCap	Multiple Regression (Ch 12)	Ch13.xls
13.1	Fixed Effects ANOVA - no interactive effects)	
13.2	Fixed Effects ANOVA - interactive effects)	
13.3	Fixed*Random Effects - Paired t-test)	
13.4	Fixed*Random Effects - Randomized Block)	
13.5	Repeated Measures, within subject	
13.6	Nested Random Effects - Hierarchical ANOVA)	
13.7	Random within Fixed (problem of confounding)	
13.8	More Than Two Factors (to be written)	

on chalk board

**ReCap** Part I (Chapters 1,2,3,4) Quantitative reasoning is based on models, including statistical analysis based on models.

**ReCap** Part II (Chapters 5,6,7)

Hypothesis testing uses the logic of the null hypothesis to declare a decision.

Estimation is concerned with the specific value of an unknown population parameter.

**ReCap** (Ch 9, 10,11) The General Linear Model with a single explanatory variable.

**ReCap** (Ch 12) GLM with more than one regression variable (multiple regression)

**ReCap** (Ch 13) GLM with more than one categorical variable (ANOVA).

Two fixed factors (Ch 13.1, Ch13.2)

Today: Special case of two factor ANOVA: Repeated Measures

One fixed and one random factor (Paired t-test, Randomized block)

**Introduction.** A very common design is to make repeated measurements of the response variable within an experimental unit of a random factor.

### **Some definitions.**

Repeated measures. An experimental unit (one level of a random factor) is measured repeatedly. Also called within subjects design

Examples.

Treatments applied in random order to each subject.

Size of fish in a tank on several occasions to obtain growth rate.

Treatments assigned randomly to adjacent subplots (2 or more).

Treatments are applied in random order, if possible, to eliminate carry-over.

A paired t-test is a special case of repeated measures.

Randomized blocks. In a standard RCBD, the blocks are groups of similar experimental units. Subjects are grouped into blocks based on a characteristic, and then treatments are randomly assigned within each block to different subjects.

Examples.

Experiment carried out in 3 plots at three locations in the intertidal zone.

Experiment carried out on several units on 4 different occasions (blocks).

Paired comparisons. Two levels of a factor within a unit (repeated measures), or in pair of units matched according to some random factor (randomized blocks).

A mixed model consists of a fixed factor of interest, and a random factor (defined unit or random block). If the random units are nested within the fixed factor, the interaction terms cannot be estimated. A two-way table of the random and fixed factors will be incomplete. As a result, the interactive effect cannot be estimated. Instead, it is folded into a new factor, a random factor within another factor.

For nested designs, the correct F-ratio is not necessarily the F-ratio formed over the residual. Here is an example, using the Flies in Cages data. It is a repeated measures design because the experimental unit (Fly) is measured repeatedly.

Random effects model Cage and Fly are random.  
Design is Random within Random

Table SS, MS, F-ratio.  
Calculate F-ratio as below

Source	df	SS	MS	F	----> p
Cage	2	665.68	332.84	1.74	0.23
Fly(Cage)	9	1720.68	191.19	146.88	>10 <sup>3</sup>
<u>Error</u>	<u>12</u>	<u>15.62</u>	1.3017		
Total	23	2401.97			

Mixed model Drug is fixed, Subj is random  
Design is Random within Fixed  
Mixed Model - Correct F-ratios

Source	df	SS	MS	F	----> p
Drug	2	665.68	332.84	1.74	0.23
Subj(Drug)	9	1720.68	191.19	146.88	>10 <sup>3</sup>
<u>Error</u>	<u>12</u>	<u>15.62</u>	1.3017		
Total	23	2401.97			

Mixed Model - Incorrect F-ratios (all ratios relative to residual)

Source	df	SS	MS	F	----> p
Drug	2	665.68	332.84	255.7	>10 <sup>3</sup>
Subj(Drug)	9	1720.68	191.19	146.55	>10 <sup>3</sup>
<u>Error</u>	<u>12</u>	<u>15.62</u>	1.3017		
Total	23	2401.97			

The simple rule is test over the random within fixed term.  
What about more complex designs?

#### 4. Calculate *LR* and *F*-ratio

Forming the likelihood ratios (and *F*-ratios) relative to the residual SS is not always correct. Here is a procedure for identifying the correct *F*-ratio. To illustrate it, we will use the Extra Sleep example.

Here is a step by step procedure to write out the correct *LR*s and *F*-ratios.

List the terms in the model, as in the ANOVA table

List the same terms horizontally. In each row, show the row term.

		<u>Drug</u>	<u>Subj%in% Drug</u>	<u>Error</u>
EMS	Drug	Drug		
EMS	Subj%in%Drug		Subj%in% Drug	
EMS	Error			Error

Each EMS includes itself

		<u>Drug</u>	<u>Subj%in% Drug</u>	<u>Error</u>
EMS	Drug	Drug		Error
EMS	Subj%in%Drug		Subj%in% Drug	Error
EMS	Error			Error

Each EMS includes the fixed error term

		<u>Drug</u>	<u>Subj%in% Drug</u>	<u>Error</u>
EMS	Drug	Drug	Subj%in% Drug	Error
EMS	Subj%in%Drug		Subj%in% Drug	Error
EMS	Error			Error

Each EMS includes crossed (or nested) random terms

		<u>Drug</u>	<u>Subj%in% Drug</u>	<u>Error</u>
EMS	Drug	Drug	Subj%in% Drug	Error
EMS	Subj%in%Drug		Subj%in% Drug	Error
EMS	Error			Error

Correct  
Denominator MS  
 Subj%in% Drug  
 Error  
 Identify the denominator MS for the *F*-ratio  
 The denominator MS cancels all but the term of interest

		<u>Drug</u>	<u>Subj%in% Drug</u>	<u>Error</u>
EMS	Drug	Drug	Subj%in% Drug	Error
EMS	Subj%in%Drug		Subj%in% Drug	
EMS	Error			Error

Incorrect  
Denominator MS  
 Error  
 Drug MS / Error results in \*two\* uncanceled terms.  
 The *F*-test is confounded.

In this example each ratio is formed relative to the term below it in the ANOVA table.

This will not always be true in more complex analyses. In these, the EMS table must be written out to identify the correct ratio