

## Model Based Statistics in Biology.

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

#### Chapter 13.5 Repeated Measures

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	Sequential within Random (Repeated Measures)	
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)

**Wrap-up.**

**Introduction.** A very common design is to make repeated measurements of the response variable within a unit of a random factor. Repeated measures can be longitudinal-repeated measures on the same individual assigned to one level of a fixed factor. Repeated measures can be cross-sectional. That is, repeated measures on an individual, with re-assignment of the individual to another level of the fixed factor. This is called a cross-over design. The major advantage of this design is that variation among units can be removed from the analysis, allowing a more sensitive test of the fixed factor of interest. Adding measurements within a unit costs less than adding units. The repeated measures design thus resembles the randomized blocks design, having the same motivation—a more sensitive analysis with lower Type II error (erroneously failing to reject the null hypothesis). The concern is that repeated measurements in time (or within a unit in space) are autocorrelated—they cannot be taken as independent estimates of the response variable.

### **Some definitions.**

Repeated measures. A unit (one level of a random factor) is measured repeatedly.

Examples.

Unit is person. Treatments applied in random order to each subject.

Unit is an animal. Only one treatment to a unit, measured repeatedly.

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

Unit is a plot. Treatments assigned randomly to adjacent subplots (2 or more).

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

Randomized blocks. A factor is applied to units that can be matched by a random factor, such as propinquity in time or space.

Examples.

Experiment carried out in 3 different labs (blocks).

Experiment carried out 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).

The general linear model will have a fixed factor of interest, and a random factor (defined unit or random block). Interaction terms cannot be estimated because fixed and random factors do not pass the cross test.

**Example** Data from

### **1. Construct model**

Verbal model.

Graphical model. Plot of

Write GLM:  $T = \beta_0 + \beta_U X_U + \beta_E X_E + \beta_{D \times S} X_D X_S + \text{residual}$   
 S&R95  $T_{i:l} = \beta_0 + \beta_U A_i + \beta_E S_i + \beta_{(A \times S)}_{i:l} + \dots$   
Formal Model

**2. Execute analysis.**

Place data in model format:

- Column labelled L, with response variable
- Column labelled X<sub>U</sub>, with explanatory variable X<sub>U</sub> =
- Column labelled X<sub>E</sub> with

Code model statement in statistical package according to the GLM

$$L = \beta_0 + \beta_U X_U + \beta_E X_E + \dots$$

```
MTB > anova 'wlength' = 'XU' 'XE';
SUBC> fits c4;
SUBC> residuals c5.
```

Here are the parameters of the model: the overall mean ( $\beta_0$ ), the mean for... and the fitted values -- the mean for each fly ( $\beta_0 + \beta_U X_U + \beta_E X_E$ ).

$$\hat{\beta}_0 = \text{mean}(L) = 24^{11} @$$

$$\hat{\beta}_0 + \hat{\beta}_E = \begin{matrix} \text{mean}(L_{\text{cage=I}}) \\ \text{mean}(L_{\text{cage=II}}) \\ \text{mean}(L_{\text{cage=III}}) \end{matrix}$$

The residuals for each measurement are computed from the means.

**3. Evaluate the model**

Plot residuals versus fitted values.

- a. No line fitted in model, so skip evaluation of straight line assumption.
- b. Homogeneity of residuals.
- c. If n small, evaluate assumptions for chisquare (t, F) distributions.

$$n = 24$$

Homogeneous?

Sum(res) = 0?

Independent?

Normal ?

**4. State population and whether sample is representative.**

**5. Decide on mode of inference. Is hypothesis testing appropriate?**

**6. State H<sub>A</sub> H<sub>0</sub> pairs, test statistic, distribution, tolerance for Type I error.**

Interaction term.

Are there more specific hypotheses about parameters? No

State test statistic                                      F-ratio  
 Distribution of test statistic                         F-distribution  
 Tolerance for Type I error                           5% (conventional level)

**7. ANOVA table: set up.**

GLM:	Y	=	$\mu_0$	+	$\mu_U X_U$	+	$\mu_E X_E$	+	res
Source:	total	=			Unit		Effect		res

Source	df	SS	MS	F	----> p
Unit					
Effect					
<u>Error</u>					
Total					

**ANOVA. Calculate df, partition according to model.**

GLM:	Y	=	$\mu_0$	+	$\mu_U X_U$	+	$\mu_E X_E$	+	res
Source:	total	=			Unit		Effect	+	res
	24 - 1	=			3 - 1	+			+ 12

Source	df	SS	MS	F	----> p
Unit	2				
Effect	9				
<u>Error</u>	<u>12</u>				
Total	23				

**7. ANOVA. Calculate variance, partition according to model.**

Calculate  $SS_{total} = 23 * \text{Var}(L) = 23 * 104.43 = 2401.98$

Here is the partitioning of the  $SS_{total}$  produced by any statistical package.

GLM:	Y	=	$\mu_0$	+	$\mu_U X_U$	+	$\mu_E X_E$	+	res
	2401.97	=			665.68	+	1720.68	+	15.62

Source	df	SS	MS	F	----> p
Unit	2	665.68			
Effect	9	1720.68			
<u>Error</u>	<u>12</u>	<u>15.62</u>			
Total	23	2401.97			

Table SS, MS, F-ratio.

Calculate  $MS = SS/df$

Source	df	SS	MS	F	----> p
Unit	2	665.68	332.84		
Effect	9	1720.68	191.19		
<u>Error</u>	<u>12</u>	<u>15.62</u>	1.3017		
Total	23	2401.97			

Calculate F ratios.

Calculate Type I error from F-distribution.

- 8. Decide whether to recompute p-value.**
- 9. Declare decision about terms.**
- 10. Report and interpret parameters of biological interest.**