# Model Based Statistics in Biology. Part III. The General Linear Model. Chapter 10.2 Two Sample t-test

ReCap.Part I (Chapters 1,2,3,4)ReCapPart II (Ch 5, 6, 7)ReCapPart III (Ch 9)10.1 Single Sample t-test10.2 Two Sample t-test10.3 One way ANOVA, Fixed Effects10.4 One way ANOVA, Random Effects

Ch10.xls Sleep data from Cushny and Peebles Daphnia ages from Sokal and Rohlf (1995) Box 9.5

on chalk board

**ReCap** Part I (Chapters 1,2,3,4)

Quantitative reasoning: Example of scallops,

which combined models (what is the relation of scallop density to substrate?) with statistics (how certain can we be?)

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

<u>Hypothesis testing</u> uses the logic of the null hypothesis to make a decision about an unknown population parameter.

Estimation is concerned with the value of an unknown parameter.

**ReCap** (Ch 9) The General Linear Model is more flexible and useful than a collection of named tests.

Regression is a special case of the GLM. We have seen an examples with the explanatory variable X fixed, with the explanatory measured with error.

Today:

Two-sample t-test as a special case of the GLM

# Wrap-up

ANOVA is a special case of the general linear model..

The explanatory variable consists of categories, which are on a nominal scale. A *t*-test is a special case of one-way (single factor) ANOVA. It contrasts two means. **GLM** *t*-test One way (single factor) ANOVA with two categories. Example. Sleep data.

1

The example will be hours of extra sleep, relative to control, for hyoscyamine (DrugA) and hyoscine-L (DrugB). Data from Cushny AR, Peebles AR (1905). The action of optical isomers. II. Hyoscines. J Physiology 32:501-510.	0.7 -1.6 -0.2 -1.2 -0.1 3.4 3.7	1.9 0.8 1.1 0.1 -0.1 4.4 5.5
The data were used by W.S. Gossett to introduce the <i>t</i> -test (Student. 1908). It was then used in the first text in statistics (Fisher 1925).	0.8	1.6 4.6 3.4
Construct model <u>Verbal model</u> : Extra time slept depends on drug. <u>Graphical model</u> : Comparison of two means.	DrugA Cushny	DrugB Z.dat
The verbal and graphical models help us distinguish response from explanatory variables. Time		: Px
<ul><li>The quantity of interest, hours of extra sleep, depends on the explanatory variable, Drug A or B.</li><li>Response variable: Hours of extra sleep T</li><li>Also called the dependent variable.</li></ul>	:	β
It has units of hours, it is on a nominal scale. I Explanatory variable: $Drug (= A \text{ or } B)$ Drug is a categorical variable. It is on a nominal scale	Drug A	В

List variables: role (response/explanatory), name, symbol, units, and type of scale.

	Variable name	<u>Symbol</u>	Units	<u>Scale</u>
Response variable	Hours of extra sleep	Т	hours	ratio
Explanatory variables	Drug	Drug		nominal

State the verbal model using names of quantities and then using symbols "Hours of extra sleep depend on drug type" Hours of extra sleep T = f(Drug type).

<u>Formal Model</u> Now write the formal model, which is what the statistical package will use to carry out the analysis. Here it is in generic notation, then in an equivalent notation specific to the data.

 $T = \beta_o + \beta_x \cdot x + \varepsilon$  generic notation  $\beta_o \text{ is the grand mean}$   $\beta_x \text{ is the difference between } \beta_o \text{ and the mean of Drug } A \text{ and of Drug } B$   $T = \beta_o + \beta_{Drug} \cdot Drug + \varepsilon$  equivalent notation  $\beta_o \text{ is the intercept, the mean for the first drug (Drug A)}$   $\beta_{Drug} \text{ is the contrast (difference) between Drug A and B}$  $\beta_0 + \beta_{Drug} = \text{mean of Drug } B$ 

2

2. Execute analysis.	Т	Drug
Data are often displayed by category, as above.	0.7	A
We reorganize the data to model format $>$	-1.6	A
1 column with response variable, extra sleep time $T$ .	-0.2	A
1 column with explanatory variable $Drug = A$ or B	-1.2	A
reoranni with explanatory variable, Drug Rord	-0.1	A
Use the formal model to code the analysis in a statistical package.	3.4	A
$T = \beta_{c} + \beta_{Drug} \cdot Drug + \epsilon$	3.7	A
1 po porug Drug C	0.8	A
MTB> ANOVA 'T' = 'Drug'	0.0	A
MTB> GLM 'T' = 'Drug'	2.0	A
	1.9	В
If you are using a graphics interface statistical package to run the	0.8	В
analysis, be sure to look at the code produced, so that you understand		В
how the model you wrote translates into a model statement in your		B
nackage		D D
pueruge.	4.4	D R
Run the stat package to obtain fitted (expected) values and residuals from	1 6	B
model parameters.	4.6	B
Fitted values Fits = E[T] = $\hat{\beta}_o + \hat{\beta}_{Drug} \cdot Drug$	3.4	B
Residuals: Res = $T - Fits$		

Here are the parameter estimates

MTB >	describe	<pre>`hrs';</pre>	by 'd	lrug <b>'</b>					
	C	lrug	N	MEAN	MEDIAN	TRMEAN	STDEV	SEMEAN	
hrs		1	10	0.750	0.350	0.675	1.789	0.566	
		2	10	2.330	1.750	2.237	2.002	0.633	

Residual and fitted values are calculated from the parameter estimates.

$\hat{eta}_0 = 0.75$	
$\hat{\beta}_{Drug} = 1.58$	$\overline{T}_{\mathrm{A}} = 0.75$
$\hat{\beta}_0 + \hat{\beta}_{Drug} = 2.33$	$\overline{T}_{ m B} = 2.33$

GLM routines produce residuals and fits as output.

Here is the model statement in R with code to produce graphical output.

GLM:  $T = \beta_0 + \beta_{Drug} \cong Drug + \varepsilon$ > Sleepmodel <- lm(T ~ Drug, data=Cushny) > plot(Sleepmodel)

#### 2. Execute analysis.

Here is a partial print-out of the data equations showing the residuals. Residuals here are offset by 1 (lag1(res) for graphical evaluation of independence of the residuals.

MTB >	print	'T'	'Drug	′′fi	lts' 'res'
ROW	T D:	rug	fits	res	lag1(res)
1	0.7	A	0.75	-0.05	5
2 ·	-1.6	A	0.75	-2.35	5 -0.05
9	0.0	A	0.75	-0.75	5 0.05
10	2.0	A	0.75	1.25	5 -0.75
11	1.9	B	2.33	-0.43	3 1.25
19	4.6	B	2.33	2.27	7 -0.73
20	3.4	B	2.33	1.07	7 2.27

#### **3a.** Evaluate the structural model.

No slopes (straight lines) used, so no straight line assumption to be checked.

**3b.** Evaluate the probability model (Normal distribution in this case). This is especially important when sample size *n* is small (less than 30 or so)

	res -			*
	-	2		
	-			*
Plot residuals vs fits	2.0+			*
1101105100015 +5 1115	-			
	-	*		*
	-			
First a server tion.	-			
First assumption:	0.0+	2		
$\Delta re$ th errors homogeneous?	-			*
The in enois nonlogeneous:	-	3		*
The residuals appear homogeneous.	-			*
	-			*
they form stacks of similarly vertical	-2.0+	*		
dismonsion anound sono	-	*		2
dispersion around zero.	-			
There is one stack for each group	-			
There is one stack for each group	-		+	fits
(each drug).		0.90	1.80	2.30
$\langle O \rangle$				

Second assumption:

Are the errors normally distributed ? The residuals here are definitely not normal.

Third assumption. Are the errors independent?

We do not know the order in which the observations were made, and so have little basis for checking this

assumption. If we assume the observations were made in the order presented in the publication, we can check the assumption by arranging the residuals in that order and then plotting residuals against their adjacent value. To do this we plot the lagged residuals against the residuals.

0.90	1.80	2.3	+Ilts 0
MTB > h	nist 're	es';	
SUBC>	incr 1.		
Histo	gram of	res	N = 20
Midpoi	.nt Co	ount	
-2.	00	5	* * * * *
-1.	00	5	* * * * *
0.	00	3	* * *
1.	00	2	* *
2.	00	2	* *
3.	00	3	* * *



other at lag 1, in the order presented in the publication.

#### Fourth assumption.

Do the residuals sum to zero? This is met whenever parameters are estimated by least squares. This assumption usually met because packages used least squares or maximum likelihood to produce unbiased estimate that sum to zero.

#### Conclusion:

Residuals are homogeneous, independent, but deviate substantially from normal.

It is interesting to note that the data used by Gossett (1908) to introduce

the *t*-test did not meet the assumptions for this test.

Because the residuals depart noticeably from normal we may need to recompute a p-value from an empirical distribution rather than from the *t*-distribution introduced by Gossett.

# 4. Partition df and SS according to model.

 $\begin{aligned} df_{\text{total}} &= \text{n-1} \qquad df_{\text{Drug}} = 2 \text{ categories} - 1 \qquad d_{\text{fresidual}} = df_{\text{total}} - df_{\text{Drug}} \\ \text{SS}_{\text{total}} &= \text{var}(T) \quad df_{\text{total}} = 4.072 \quad 19 = -77.37 \\ \text{SS}_{\text{residual}} &= \sum \left(T - \overline{T_{DrugA}}\right)^2 + \sum \left(T - \overline{T_{DrugB}}\right)^2 \\ \text{SSDrug} &= \text{SS}_{\text{total}} - \text{SS}_{\text{residual}} = 12.48 \end{aligned}$  This is the improvement in fit.

GLM:	$T - \beta_o$	=	$\beta_{Drug}$ Drug	+	Е
Source:	Total	=	Drug		residual
df	20 - 1	=	1	+	18
SS	77.37	=	12.48	+	64.89

# 4. How good is the evidence?

# Calculate likelihood ratio for the overall (omnibus) model.

 $LR = (64.89/77.37)^{-20/2} = 5.8$ 

LR < 20 There is insufficient evidence of a difference between the 2 means.

In the absence of good evidence (LR > 20), or even some evidence (LR > 10) evidentialist inference is appropriate.

# 4. Decide on mode of inference.

However, controlling Type I error is relevant if the drug is being considered for use with people. So Type I error (risk of false positive) is relevant to reporting the experimental results. We use a frequentist approach to make a decision relevant to Type I error.

# 5. State sample and population for frequentist inference.

The population in this case: an infinitely large number of repeats of the same experiment with the same drug. The term  $\beta_{Drug}$  in the model is the long run difference between the two means. We could estimate this parameter for the population by running the experiment repeatedly, then taking the average value of the differences between the two groups. The sample is considered applicable to any repeat of the experiment conducted in the same way.

# 6. For frequentist inference, state $H_A$ / $H_0$ pair, test statistic, its distribution, and tolerance of Type I error.

There is one term in the model. Is this term significant ? (not due to chance).

The research hypothesis is that the drugs differ in effect.	H <sub>A</sub> : $\mu_A \neq \mu_B$
The null hypothesis is the drugs do not differ in effect.	H <sub>o</sub> : $\mu_A = \mu_B$

Here is an equivalent formulation.

The research hypothesis is that the drugs differ in effect.	H <sub>A</sub> : $\beta_{Drug} \neq 0$
The null hypothesis is the drugs do not differ in effect.	H <sub>o</sub> : $\beta_{Drug} = 0$

The symbol  $\beta_{Drug}$  has a single value, the difference between the two means. The hypotheses listed above are equivalent to the following pair of hypotheses If the means differ, then  $\operatorname{var}(T_A - T_B) > 0$ If the means are the same, then  $\operatorname{var}(T_A - T_B) = 0$ H<sub>o</sub>:  $\operatorname{var}(T_A - T_B) = 0$ 

State test statisticF ratio or t- statistic. Note:  $t^2 = F$ .Distribution of test statisticF distributionTolerance for Type I error $\alpha = 5\%$ 

The tolerance for Type I error is called  $\alpha$ , which is conventionally set at 5%. This is a compromise between Type I and II error. One can set this at 1% or at 10%, depending on whether one is worried about Type I or II error. Setting  $\alpha$  at a low value increases Type II error, the chance of rejecting a true effect. If many tests are to be made, it is advisable to set tolerance for Type I error at  $\alpha/n$  where n = number of tests. This is called a Bonferroni criterion, for Bonferroni tests.

It takes into account the fact that the Type I error for multiple tests is not the same as for a single test. If you set  $\alpha = 5\%$  and perform 20 tests, then you expect one "significant" result even when there is no real effect.

Because  $\alpha$  should generally be set before one carries out a test  $\alpha$  should be stated in the methods section. If different  $\alpha$  levels are used in a single study, the reasons for the different levels should be stated.

#### 7. ANOVA - Move df and SS to ANOVA table.

Source	df	SS	MS	F	> p
Drug	1	12.48			
Res	18	64.89			
Total	19	77.37			

MS stands for the mean squared deviation. MS = SS / df

 $MS_{model} = MS_{Drug} = 12.482$ 

 $MS_{res} = MS_{error} = 3.605$ 

 $MS_{tot} = Var(response) = Var(T)$ 

MS from SS and df in ANOVA table F from MS p from F distribution

Calculations move from left to right,

 $MS_{tot}$  does not appear in the MS column, because  $MS_{model} + MS_{res} \neq MS_{tot}$ 

7. ANOVA - (	Compute test statisti	c F = (MS)	$S_{Drug})/(MS_{res})$	$= (SS_{Drug})/$	$(SS_{total} - SS_{Drug})$	
	Source	df	SS	MS	F> p	
	Drug	1	12.48	12.482	3.46	
	Res	<u>18</u>	64.89	3.605		
	Total	19	77.37			

The *F*-ratio can be thought of as the signal to noise ratio.

How strong is the signal, relative to the noise (error)?

F is the ratio of the explained variance (due to the entire model,

or due to a factor in the model) to the unexplained variance.

E.g.,  $F = MS_{model} / MS_{res}$ 

This can be calculated by hand, if necessary, using MS or SS from computer package. Forming the correct F-ratio can require considerable skill and experience,

especially with complex experimental designs.

Computer packages sometimes produce incorrect F-ratios. It is a good idea to check with a statistician, if in doubt.

The completed table represents a sequence of computations from left to right. It results in an F-ratio, which will be small if the explained variance is small, large if the MS<sub>Drug</sub> is large

# 7. ANOVA Table source, df, SS, MS, F-ratio, and p-value.

Here are the ANOVA calculations in a spreadsheet.

	Time	Drug	Fits	Residuals		
	0.7	Ō	0.75	-0.05		
	-1.6	0	0.75	-2.35		
	-0.2	0	0.75	-0.95		
MTB > cdf $3.46;$	-1.2	0	0.75	-1.95		
SUBC> f 1 18	-0.1	0	0.75	-0.85		
0 921 3 46	3.4	0	0.75	2.65		
0.721 5.40	3.7	0	0.75	2.95		
	0.8	0	0.75	0.05		
	0	0	0.75	-0.75		
	2	0	0.75	1.25		
	1.9	1	2.33	-0.43		
	0.8	1	2.33	-1.53		
	1.1	1	2.33	-1.23		
	0.1	1	2.33	-2.23		
	-0.1	1	2.33	-2.43		
	4.4	1	2.33	2.07		
	5.5	1	2.33	3.17		
	1.6	1	2.33	-0.73		
	4.6	1	2.33	2.27		
	3.4	1	2.33	1.07		
	4.072		0.657	3.415	variance	
	77.368	=	12.482	64.886	SS=	19*variance
	19.000		1.000	18.000	df	
			12.482	3.605	MS	

$$\begin{split} SS_{tot} &= Var(T)^* \ df_{tot} \\ SS_{fits} &= Var(fits) * \ df_{tot} \\ SS_{res} &= Var(res) * \ df_{tot} \\ \end{split}$$
 The same computations can be carried out in any package

# 7. Calculate p-value for terms in model.



.

Hence p = 1 - .921 = 0.079

Source	df	SS	MS	F>	р
Drug	1	12.48	12.482	3.46	0.079
Res	18	77.37	3.06		
Total	19	64.89			

#### 8. Recompute p-value if necessary.

When assumptions not met, recompute if: n small (Yes, n = 20)

```
p near \alpha (Yes, p =0.079)
```

Because p-value is near  $\alpha$ . the decision might change if the p-value recomputed by randomization.

Colquhoun (1971) carried out a randomization test, using

12000 of the 184,756 possible permutations of the data into 2 groups.

The p-value was p = 0.0813 (976/12000)

This is close to p-value from t-test, and it leaves the decision unchanged.

The p-value changed by a factor of 0.0813 / 0.079 = 1.03 (hardly at all) We find that the substantial violation of normal error assumption had little effect on the estimate of Type I error (the p-value) in this case. This is because the distribution was symmetrical around the mean, despite the deviation in shape.

Note: Usually it is not feasible to construct frequency distribution from all permutations. Instead, we sample from the list of all permutations by sampling at random from the data, and computing the F-ratio repeatedly to construct the frequency distribution of F when the null hypothesis is true.

Computer packages produce ANOVA tables with F-ratios and p-values. However, it is important to learn how one quantity is computed from another in this table, in order to understand the table. It is also important to write the model out, before executing the analysis. Writing the model, and the list of explanatory variables, then calculating the degrees of freedom, is useful in making sure the computer executed the analysis you had in mind, rather than something else.

# 9. Declare and report decision about model terms (compare *p* to α).

*I.e.* Compare the observed statistic to population of such statistics.  $0.0813 = p > \alpha = 0.05$  So we cannot reject H<sub>o</sub>: Var( $\beta_{Drug}$ ) = 0

Equivalently, cannot reject  $H_0$ :  $\beta_{Drug} = 0$ 

Report decision and conclusion:

Decision: We cannot reject the null.

Conclusion: There is no statistically significant difference in extra time slept.  $F_{1,18} = 3.46 \ p = 0.081$  (randomized)

When we cannot reject the null hypothesis, we then consider Type II error, that of a null hypothesis that is not true.

We ask: What difference could have been detected, given the variance and the sample size? To answer this, we take the observed difference between the means ( $\Delta T = 2.33 - 0.75 = 1.58$  hours), then increase this difference until the p-value becomes significant. We start with guess: we increase difference by adding 0.5 hours to each value in the group with the larger mean. This increases the mean to 2.83 hours. It increases the difference to  $\Delta T = 2.08$  hours. Then we run the GLM routine to obtain the p-value.

#### 9. Report decision about model terms (continued)

We run the GLM routine repeatedly until we find the difference that results in p = 0.05.  $\Delta T = 2.33 - 0.75 = 1.58$  F = 3.46 p = 0.079 p-value from F-distribution  $\Delta T = 2.83 - 0.75 = 2.08$  F = 6.00 p = 0.025 too high, try 0.2 increase  $\Delta T = 2.53 - 0.75 = 1.78$  F = 4.39 p = 0.05

The minimum detectable difference was 1.78 hours, which is higher than the observed difference by 1.78/1.58 = 1.127. With this sampling effort and variance we could have detected a difference of 1.78 hours. A true difference of 1.77 hours of extra sleep would go undetected by this experiment. A better experiment is needed, one that has a chance of detecting a smaller difference. One way to improve the experiment is to increase the number of trials, which will reduce the error variance.

If we are planning another experiment it is informative to compute the sample size needed to detect a difference, given the variance and contrast between means. To do this we increase sample size until the F-ratio becomes significant at 5%. Because p is already close to  $\alpha$  we start with a small increase of 10, from n = 20 to n = 30.

Try a slightly smaller increase, of 8 (4 per group), from n = 20 to n = 28

Source	df	SS	MS	F>	р
Drug	1	12.48	12.482	4.19	0.051
Res	26	77.37	2.976		
Total	27	64.89			

Assuming the same variance and same difference in means, a sample size of 15 per group

(n = 30) was needed to detect the observed difference. This is a feasible increase. These calculations are readily done in a spread sheet that recalculates from a change in the total df.

# 10. Report and interpret parameters of biological interest.

The estimated effect size was 2.33 - 0.75 hours = 1.58 hours. To illustrate the t-test, Gossett chose an example where the magnitude of the effect size, by itself, would lead to a conclusion of a substantial difference between the two drugs. The effect size was large but the variability did not allow us to reject the null hypothesis at a conventional 5% Type I error rate. With this sampling effort and variability, we could have detected a difference of 1.78 hours in time of sleep, which is only 13% higher-- 1.78/1.58 = 1.13. An effect smaller than 1.78 hours would go undetected with this sampling effort and variability. The study needs to be repeated to be conclusive.

#### Example. Daphnia ages

Data from Box 9.5 p 220 Sokal and Rohlf 1995 Does time to maturity differ in two genetic crosses in the water flea *Daphnia* ?

#### 1. Construct model

Verbal model: age depends on strain.

Graphical model.

Draw means at 7.56 (Strain 1) and at 7.51 (strain 2)

Response variable

A = age (in days) at beginning of reproduction in

*Daphnia longispina* in two genetic crosses I and II (ratio type of scale) Explanatory variable.

 $St = I \text{ or } II \pmod{n}$  (nominal scale) n = 14 observations, 7 in each of groups I and II

Formal Model  $A = \beta_o + \beta_{St} \cdot St + \varepsilon$ 

 $\beta_o$  is the intercept (mean of reference group, Strain I).

 $\beta_{St}$  is the contrast (dfference) between the two groups

 $\beta_o + \beta_{St} \cdot St$  = mean of second group Strain II

#### 2. Execute analysis. Place data in model format:

Column with response variable, Age A.

Column with explanatory variable, Strain = I or II

Code model statement in statistical package according to the GLM

```
A = \beta_o + \beta_{St} \cdot St + \varepsilon

MTB> ANOVA 'A' = 'Strain'

MTB> GLM 'A' = 'Strain';

SUBC> fits c3;

SUBC> res c4.

MTB > plot c4*c3
```

Code for R

> Daphniamodel <- lm(A ~ St, data=Daphnia) > plot(Daphniamodel)

# parameters reported by GLM routine

 $\hat{\beta}_0 = 7.5571 = \text{Strain 1 mean, the intercept}$  $\hat{\beta}_{St} = 0.0428 = \text{Contrast, the difference between the two means.}$ 

#### **3a.** Evaluate structural model

No slopes (straight lines) used, so no need to check for bowls/arches.



# **3b.** Evaluate error model model

#### Homogeneous?

MTB

\_ -1.60+

-1.50

The two stacks of residuals in this plots are of similar spread, so we conclude th a acidual

	the resi	duais are no	mogeneous	5.			
	MTB > plot	'res' 'fit	s'				
	-						
	0.80+						*
	-	*					
	res =	â					*
	-	*					*
	0.00+	*					2
	-						*
	-						
	-						
	-						
	-0.80+						
	-						
	_						*
	-						
	-1.60+	*					
		+_	+	+	+	+	fits
		7.5200	7.5280	7.5360	7.5440	7.5520	
					MTB > his	t 'res'	
						-	
	<u>Normal?</u>				Histogram	of res N	= 14
	No, res	iduals area s	kewed tow	ard	Midpoint	Count	
	negativ	e residuals l	ov outliers		-1.6	1 *	
	negutiv	e residuais e	y outliers.		-1.2	1 *	
		4			-0.8	0	
	Independe	<u>nt?</u> Plot res	siduals at la	ıg 1.	-0.4	U 5 ++-	* * *
	No	evident up o	r down trer	nd.	0.0	6 ***	* * * *
	Res	iduals indae	d to be ind	enendent	0.8	1 *	
				pendent.			
TIB >	> piot · res ·	'lagres'					
	0.80+				*		
	_						
res	-				*		
	_ *	5			*	*	
	-			-	* *		
	0.00+	*		*	*		
	-				*		
	_						
	_						
-	-0.80+						
	_						
	_						
	-				*		

0.00

-0.50

-1.00

\*

0.50

---+----+lagres

1.00

#### **3b.** Evaluate probability model

Conclusion. Residuals are homogeneous and independent, but deviate from normal .

#### 4, Partition df and variance according to model.

```
ANOVA table headings on chalk board, upper right.
GLM just to the left.
Headings under model, then move to ANOVA table
```

Calculate  $df_{total} = n - 1 = 14 - 1 = 13$  Partition df according to model Calculate SS<sub>tot</sub> from Var(*A*), the variance of response variable.  $SS_{tot} = Var(A) \cdot df_{tot} = 0.42247 \cdot 13 = 5.49214$ 

Use statistical package to partition SS<sub>tot</sub> according to model

GLM:	$A - \beta_o$	=	$\beta_{St} \cdot St$	+	Е
Source:	Total	=	Strain+	resi	dual
df	13	=	12	+	1
SS	5.492	=	0.00641	+	5.4857

# 4. How good is the evidence for a difference?

Calculate the likelihood ratio for model.

 $LR = (5.48571/5.492)^{-14/2} = 1.008$ 

LR < 10 There is no evidence of a difference.

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

In the absence of any evidence for a difference, evidentialist inference is appropriate. However, the likelihood ratio was calculated assuming a normal error, which was not warranted by examination of the histogram of the residuals. We could recompute the likelihood using randomization in place of assuming a normal error (Owen text).

# **10.** Report and interpret parameters of biological interest.

$\overline{A}_{I} = 7.5571 \text{ days}$	stdev = $1.319$ days	<i>n</i> = 7
$\overline{A}_{II} = 7.5143 \text{ days}$	stdev = $1.976$ days	<i>n</i> = 7

The two means differ by only 6 parts in a 1000 (7.5571 - 7.5143)/7.5357 = 0.006The parameter of biological interest is the average time to maturity, regardless of strain, which is  $\overline{A} = 7.5357$  days stdev = 1.997 days n = 14There is no evidence of any difference in time to maturity between the two strains.

Was the lack of evidence due to poor execution? To address this we look at the minimum difference that could have been detected, given the variance and sample size.

To do this we keep increasing the difference between two groups until the difference reaches a threshold, such as a Type I error of 5%. In practice we add an offset to one group, compute the t-statistic and p-value, increase the offset, compute the t-statistic and p-value gain, and continue until the p-value falls below the significance level (5%).

#### 10. Report and interpret parameters (continued)

This computation can be done in a spreadsheet.

1				1	
Age	Strain	Fits	Residuals	Strain 0	Offset
8.002	0	8.3163	-0.31429	7.2	0.802
7.902	0	8.3163	-0.41429	7.1	0.802
9.902	0	8.3163	1.585714	9.1	0.802
8.002	0	8.3163	-0.31429	7.2	0.802
8.102	0	8.3163	-0.21429	7.3	0.802
8.002	0	8.3163	-0.31429	7.2	0.802
8.302	0	8.3163	-0.01429	7.5	0.802
8.8	1	7.5571	1.242857		
7.5	1	7.5571	-0.05714		
7.7	1	7.5571	0.142857		
7.6	1	7.5571	0.042857		
7.4	1	7.5571	-0.15714	Strain 0	8.3163
6.7	1	7.5571	-0.85714	Strain 1	7.5571
7.2	1	7.5571	-0.35714	Diff	0.7591
0.577		0.155	0.422	variance	
7.503	=	2.017	5.486	SS=	13*variance
13.000		1.000	12.000	df	
		2.017	0.457	MS	
			4.412281	F	
			0.050037	α	

The two strains would have to differ by 0.76 days to reach the 5% Type I error threshold. *I.e.* the strains would have to differ by (0.7591/7.5571) = 10% to be significant.

The analysis was capable of detecting a 10% difference in age.

The absence of evidence for a difference cannot be attributed to a poorly executed study.

#### Extra

The t-test is a special case of a one-way ANOVA. The *F*-ratio, by definition, is  $t^2$ 

ere	is the ANO	VA lable	calculation	1  of the  F-r	allo	
	Source	df	SS	MS	F	
	strain	1	0.00641	0.00641	0.014	
	error	12	5.48571	0.45714		

Here is the ANOVA table calculation of the *F*-ratio

Here is the calculation of the F- ratio from the formula for the *t*-statistic.

$$t = \frac{\left(\overline{X}_{I} - \overline{X}_{II}\right) - \left(\mu_{I} - \mu_{II}\right)}{\sqrt{\frac{1}{n}\left(S_{I}^{2} + S_{II}^{2}\right)}} \qquad t = \frac{\left(7.5571 - 7.5143\right) - \left(0 - 0\right)}{\sqrt{\frac{1}{7}\left(0.50476 + 0.40952\right)}} = \frac{0.4286}{\sqrt{\frac{0.9143}{7}}} = \frac{0.4286}{0.3614} = 0.1186$$
$$t^{2} = 0.1186^{2} = 0.014$$