58I

Quantitative and Computational skills

58I Lab and Prof Skills II

Lecture Overview

Introduction to Q&C skills strand

- Q&C skills strand in 58I
- Data Skills in degree program roadmap

Stage 1 - revision, brief!

Linear models - what are they?

Revisiting regression, t-tests and ANOVA as linear models

Learning Objectives for 58I as a whole

- 1. To be able to generate a testable hypothesis.
- 2. To design and conduct experiments to test this hypothesis, with appropriate controls.
- 3. To have practical experience of a range of techniques relevant to the discipline.
- 4. To work effectively within a team.
- 5. To be able to write a scientific report based on practical work.
- 6. To communicate scientific information and ideas in the form of a variety of media to a variety of audiences.
- 7. To use appropriate graphical methods to produce data figures with appropriately detailed legends.
- 8. To use relevant statistical or other analytical methods to analyse data.
- 9. To research scientific literature in a given area, and write an extended and well-structured account.

Learning Objectives for 58I Q&C

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- 9. To research scientific literature in a given area, and write an extended and well-structured account.

Assessment

Express competency in Experimental Design and Bioscience Techniques (and elsewhere)

Becoming Competent

Make it fun. Practice and engage with people.

The workshops are not tests, they are opportunities.

It is expected that you make a lot of mistakes and need help.

Talk to each other, demonstrators and lecturers.

"There are two ways to write error free code and only the third way works"

Topics covered in 58I Q&C

Impossible to cover everything to you might ever need!

Different people will use different topics

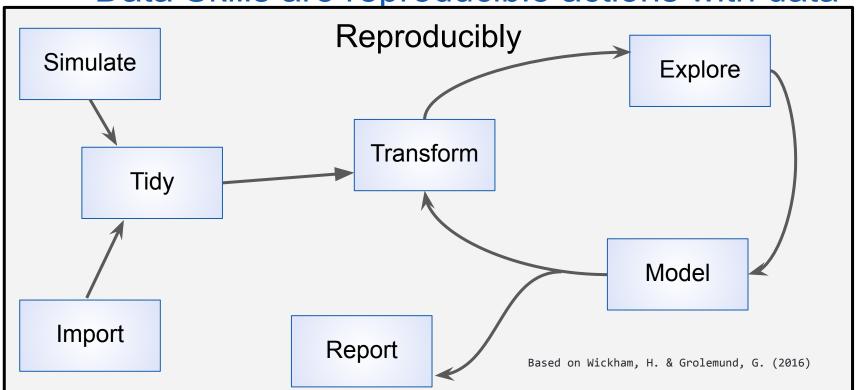
Chosen topics are: foundational, follow stage 1 well, widely applicable (in this module and beyond), transferable conceptually:

- Generalised Linear Models:
- Non-linear Models (non-linear regression)

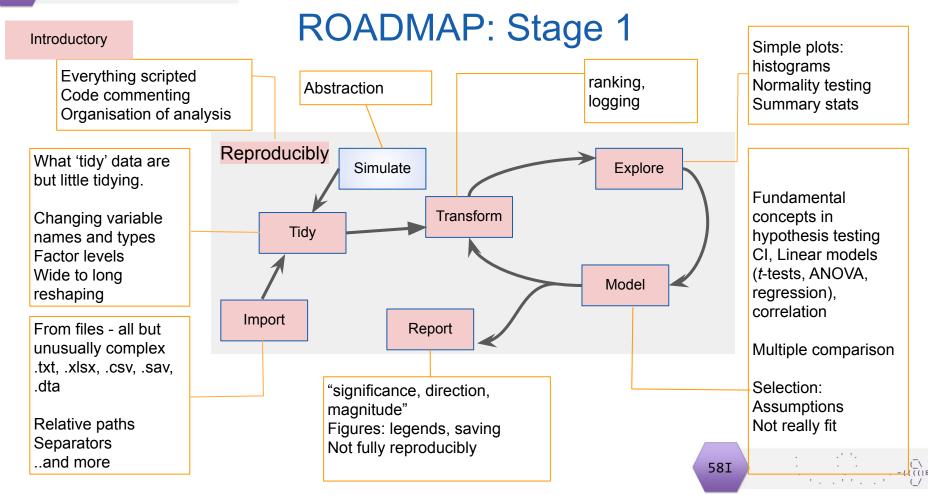
Methods which are very specific to the Experimental Design / Bioscience Technique taken are covered in that option. Talk to your project leader.

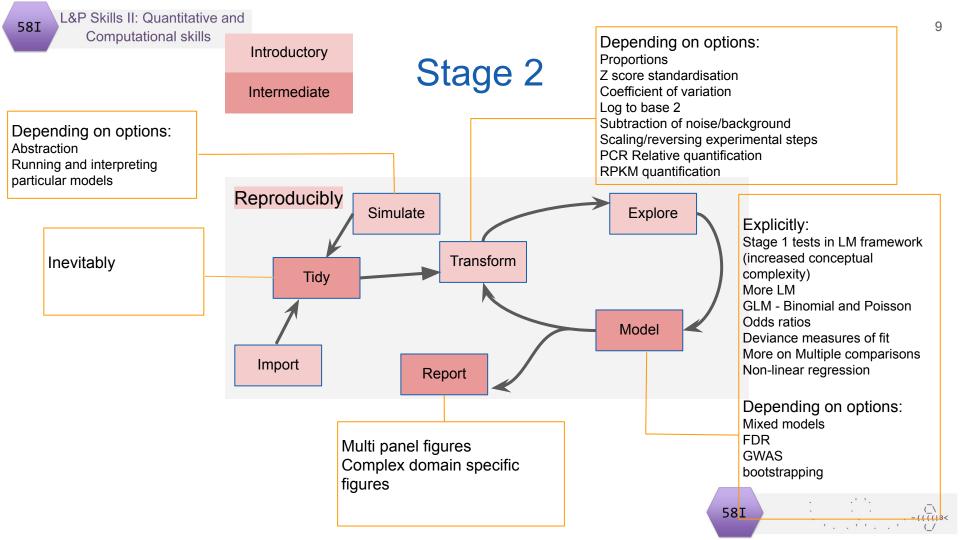
58I

Data Skills are reproducible actions with data

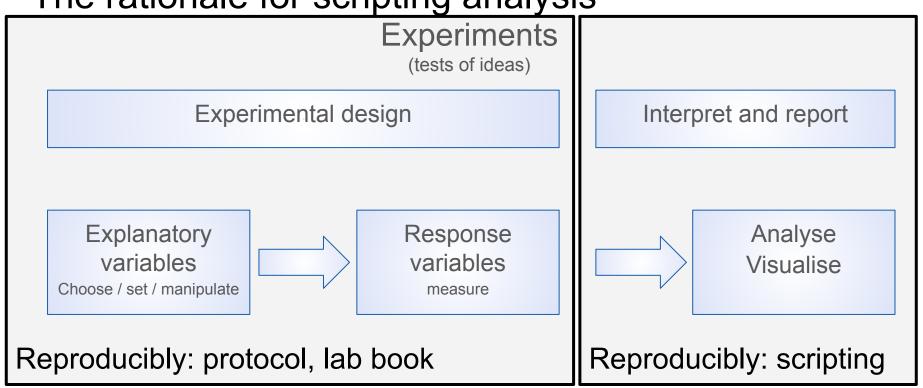


L&P Skills II: Quantitative and Computational skills





The rationale for scripting analysis

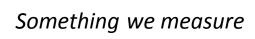


Why R?

It's a good choice but not the only option.

- R caters to "users who do not see themselves as programmers, but then allows them to slide gradually into programming"
- Community, active, relatively diverse
- Language designed for data analysis and visualisation so makes those easy
- Open source, Free,
- Reproducibility R markdown, R's "killer feature"

Stage 1 Revision: experiments and analysis



Can be explained by

Some things we control, choose or set

Response variable

Dependent variable The 'v' s Relationship

Predictor variables

Independent variable(s)
The 'x' s



function(y $\sim x$) function(y $\sim x_1 * x_2$) 58I

Stage 1 Revision: experiments and analysis

Something we measure

Can be explained by

Some things we control, choose or set

Response variable

Normally distributed

Relationship

Linear

Predictor variables

Continuous: regression

Categories: t-test, ANOVA



function(y $\sim x$) function(y $\sim x_1 * x_2$)

Contact time: 1 lecture + 4 workshops

Lecture 1: Introduction to Generalised Linear Models (ER)

Workshop 1: Linear Models (ER)

T-tests, ANOVA and regression are used when we have a continuous response variable. We revisit these using a linear modelling framework. This means using a single function `lm()` rather than three different ones and enhancing our understanding of the concepts underlying the tests.

Workshop 2: Generalised Linear Models for Poisson distributed data (ER)

Workshop 3: Generalised Linear Models for Binomially distributed data (ER) We extend our knowledge of linear models by considering other types of response variable.

Workshop 4: Non-linear regression and dynamics (JWP)

Lecture Overview

Introduction to Q&C skills strand

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- Data Skills in degree program roadmap

Stage 1 - revision, brief!

Linear models - what are they?

Revisiting regression, t-tests and ANOVA as linear models

Learning objectives

By actively following this lecture and undertaking the exercises in workshop 1 the successful student will be able to:

- Explain the the link between t-tests, ANOVA and regression
- Appropriately apply linear models using lm()
- Interpret the results using summary() and anova() and relate them to the outputs of t.test() and aov()

What are linear models?

Something you have already met!

Equation to explain, with a linear relationship, one response variable with one or more explanatory variables: $y = ax_1 + bx_2 +$

Procedure	Response	Explanatory	R	Stage 1 examples
Single linear regression	Continuous	1 Continuous	y~x	mand ~ jh mass ~ day
Two-sample t-test	Continuous	1 categorical (2 levels)	y ~ x	adiponectin ~ treatment time ~ status
One-way ANOVA	Continuous	1 categorical (2 or more levels)	y~x	myoglobin ~ species
Two-way ANOVA	Continuous	2 categorical (2 or more levels each)	y~x1*x2	para ~ season * species diameter ~ agent * species

Key points

T-tests, ANOVA and regression are fundamentally the same, collectively called 'general linear models'. They can be carried out in R with 1m()

There are other linear models too

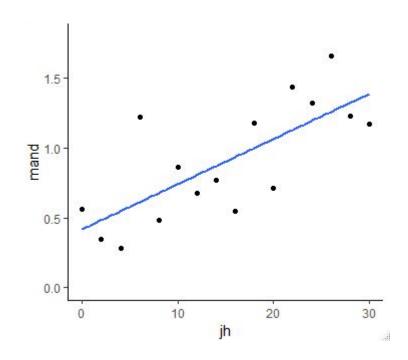
The concept can be extended to 'generalised linear models' for different types of response. Generalised linear models are carried out in R with glm()

The output of lm() looks more complex, at first, than the outputs of t.test() and aov()

The output of glm() is like that for lm(). So we will revisit regression, t-tests and ANOVA using lm() to help you understand the output

Concentration of juvenile hormone (JH) and mandible length in stag beetles

```
mod <- lm(data = stag, mand ~ jh)</pre>
```



```
mod <- lm(data = stag, mand ~ jh)</pre>
summary(mod)
Call:
lm(formula = mand ~ ih, data = stag)
Residuals:
   Min
         10 Median
                               Max
                         30
-0.38604 -0.20281 -0.09751 0.15034 0.60690
Coefficients:
         Estimate Std. Error t value Pr(>|t|)
jh
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 0.292 on 14 degrees of freedom
```

Multiple R-squared: 0.5429, Adjusted R-squared: 0.5103

F-statistic: 16.63 on 1 and 14 DF, p-value: 0.00113

```
y = 0.42 + 0.03*jh
mod <- lm(data = stag, mand ~ jh)</pre>
   summary(mod)
                                                                       Intercept
   Call:
   lm(formula = mand ~ ih, data = stag)
                                                                       Slope
   Residuals:
        Min
                  10 Median
                                     30
                                              Max
                                                                       Test of intercept
   -0.38604 -0.20281 -0.09751 0.15034
                                         0.60690
                                                                       Test of slope
   Coefficients:
   Estimate Std. Error t value Pr(>|t|) (Intercept) 0.419338 0.139429 3.008 0.00941 **
                                                                       % of variation in y explained by x
               0.032294 0.007919 4.078 0.00113 **
   jh
                                                                       "model fit"
   Signif. codes: 0 '***' 0.001 '**' 0.05 .' 0.1 ' ' 1
   Residual standard error: 0.292 on 14 degrees of freedom
                                                                       Test of model
   Multiple R-squared: 0.5429, Adjusted R-squared: 0.5103
   F-statistic: 16.63 on 1 and 14 DF, p-value: 0.00113
                                                                                   58I
```

Revisiting: Regression - thi

Intercept

```
mod <- lm(data = stag, mand ~ jh)</pre>
```

summary(mod)

Call:

 $lm(formula = mand \sim jh, data = stag)$

Residuals:

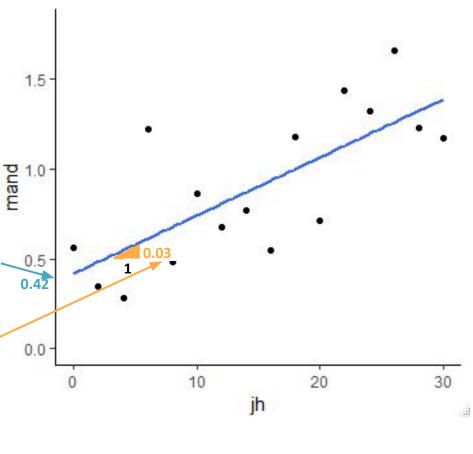
Min 1Q Median 3Q Max -0.38604 -0.20281 -0.09751 0.15034 0.60690

Coefficients:

Estimate Std. Error t value Pr(>|t|) (Intercept) 0.419338 0.139429 3.008 0.00941 jh 0.032294 0.007919 4.078 0.00113

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '

Residual standard error: 0.292 on 14 degrees of freedom Multiple R-squared: 0.5429, Adjusted R-squared: 0.5103 F-statistic: 16.63 on 1 and 14 DF, p-value: 0.00113



```
When only one continuous
mod <- lm(data = stag, mand ~ jh)</pre>
                                            variable after the ~
  summary(mod)
  Call:
  lm(formula = mand ~ ih, data = stag)
  Residuals:
       Min
              10 Median
                               30
                                      Max
  -0.38604 -0.20281 -0.09751 0.15034 0.60690
  Coefficients:
             Estimate Std. Error t value Pr(>|t|)
  0.007919 4.078 0.00113 ***
             0.032294
  jh
  Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 '
  Residual standard error: 0.292 on 14 degrees of freedom
  Multiple R-squared: 0.5429, Adjusted R-squared: 0/.5103
  F-statistic: 16.63 on 1 and 14 DF, p-value: 0.00113
```

P value for slope of single variable = P value of whole

P value of whole model

This will not be true for more for i) one-way anova with more than 2 gps ii) two-way anova iii) other linear models

Revisiting: two-sample t-test using t.test()

 $t.test(y \sim x, data = mydata, var.equal = T)$

Example 1 from 17C.

Is there a significant difference between the masses of male and female chaffinches?

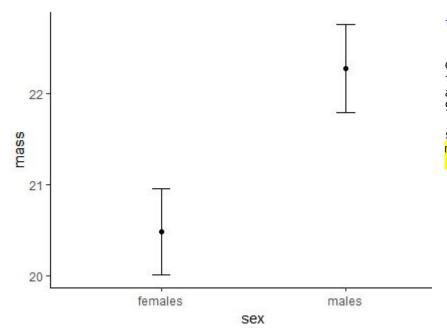
Example 2 from 08C.

Does treatment with Nicotinic acid affect adiponectin secretion compared to control treatment?

```
t.test(mass ~ sex, data = chaff, var.equal = T)
      Two Sample t-test
data: mass by sex
t = -2.6471, df = 38, p-value = 0.01175
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -3.167734 -0.422266
sample estimates:
mean in group females
                      mean in group males
               20,480
                                     22,275
t.test(adiponectin ~ treatment, data = adip, var.equal = T)
       Two Sample t-test
       adiponectin by treatment
t = -3.2728, df = 28, p-value = 0.00283
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -3.1910762 -0.7342571
sample estimates:
  mean in group control mean in group nicotinic
                                       7.508667
               5.546000
```

Revisiting: two-sample t-test using t.test()

 $t.test(y \sim x, data = mydata, var.equal = T)$



Alternative way to state:

different

Sex has a significant effect on mass

Using t.test

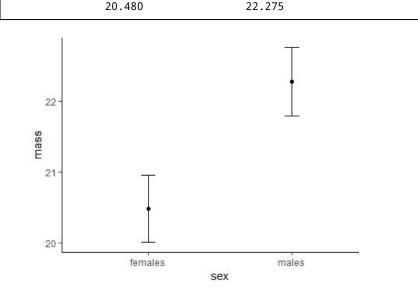
Revisiting: Comparing t.test() with Im()

```
t.test(mass ~ sex, data = chaff, paired = F, var.equal = T)

Two Sample t-test
data: mass by sex
t = -2.6471, df = 38, p-value = 0.01175
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
    -3.167734 -0.422266
sample estimates:
mean in group females mean in group males
```

Output of Im() to do a t-test looks the same as the output of Im() to do a regression.

Mathematically the same thing!



```
mod <- lm(mass ~ sex, data = chaff)</pre>
summary(mod)
Call:
lm(formula = mass ~ sex, data = chaff)
Residuals:
    Min
             10 Median
                                    Max
-5.2750 -1.7000 -0.3775 1.6200 4.1250
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 20.4800
                         0.4795 42.712
                                          <2e-16 ***
                                                               Difference is
              1.7950
                         0.6781
                                          0.0118 *
sexmales
                                                              significant
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 2.144 on 38 degrees of freedom
Multiple R-squared: 0.1557, Adjusted R-squared: 0.1335
F-statistic: 7.007 on 1 and 38 DF, p-value: 0.01175
```

L&P Skills II: Quantitative and Computational skills

t.test(mass ~ sex, data = chaff, paired = F, var.equal = T)

Using t.test

Revisiting: Comparing t.test() with Im()

```
Two Sample t-test
                                                                                               Intercept is mean of 'lowest' level of factor
data: mass by sex
t = -2.6471, df = 38, p-value = 0.01175
alternative hypothesis: true difference in means is not equal to 0
                                                                                               I.e., equivalent to x = 0 in regression
95 percent confidence interval:
-3.167734 -0.422266
sample estimates:
mean in group females
                        mean in group males
               20.480
                                     22.275
                                                                                                                                       Using Im()
                                                                     mod <- lm(mass ~ sex, data = chaff)</pre>
                                                                     summary(mod)
                                                                     Call:
                                                                                                                          Female mean sig diff
                                                                     lm(formula = mass ~ sex, data = chaff)
                                                                                                                          from 0. Not important
                                                                     Residuals:
                                                                                  10 Median
                                                                         Min
                                                                                                         Max
                                                                     -5.2750 -1.7000 -0.3775 1.6200 4.1250
                                                                     Coefficients:
                                                                                 Estimate Std. Error t value Pr(>|t|)
                                                                     (Intercept) 20.4800
                                                                                              0.4795 42.712
                                                                                                               <2e-16 **
                                                                     sexmales
                                                                                   1.7950
                                                                                              0.6781
                                                                                                       2.647
                                                                                                               0.0118 *
                                                                     Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
     20
                                                                     Residual standard error: 2.144 on 38 degrees of freedom
                                                                     Multiple R-squared: 0.1557, Adjusted R-squared: 0.1335
                   females
                                          males
                                                                     F-statistic: 7.007 on 1 and 38 DF, p-value: 0.01175
                                sex
```

L&P Skills II: Quantitative and Computational skills

Using t.test

Revisiting: Comparing t.test() with Im()

```
t.test(mass ~ sex, data = chaff, paired = F, var.equal = T)
                                                                                                  Difference between intercept
       Two Sample t-test
data: mass by sex
                                                                                                  and next level (i.e., the slope)
t = -2.6471, df = 38, p-value = 0.01175
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
                                                                                                  I.e., Changing x by 1 unit
-3.167734 -0.422266
                                                                                                  makes y go up by the value of
sample estimates:
mean in group females
                        mean in group males
                                                                                                  slope
               20.480
                                     22.275
                                                                                                                                     Using Im()
                                                                    mod <- lm(mass ~ sex, data = chaff)</pre>
                                                                    summary(mod)
                                                                    Call:
                                                                    lm(formula = mass ~ sex, data = chaff)
     22
                                                                    Residuals:
                                                                                 10 Median
                                                                        Min
                                                                                                        Max
                                                                    -5.2750 -1.7000 -0.3775 1.6200 4.1250
                                                                    Coefficients:
                                                                                Estimate Std. Error t value Pr(>|t|)
                                                                    (Intercept) 20.4800
                                                                                             0.4795 42.712
                                                                                                              <2e-16 ***
                                                                                                                                   Difference is
                                                                                  1.7950
                                                                                             0.6781
                                                                                                              0.0118 *
                                                                    sexmales
                                                                                                                                  significant
                                                                    Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ''
     20
                                                                    Residual standard error: 2.144 on 38 degrees of freedom
                                                                    Multiple R-squared: 0.1557, Adjusted R-squared: 0.1335
                   females
                                          males
                                                                    F-statistic: 7.007 on 1 and 38 DF, p-value: 0.01175
                               sex
```

Why use Im()?

Extendable! These are particular cases but a linear models include any number of continuous and categorical explanatory variables.

Procedure	Response	Explanatory	R	Stage 1 examples
Single linear regression	Continuous	1 Continuous	y~x	mand ~ jh mass ~ day
Two-sample t-test	Continuous	1 categorical (2 levels)	y ~ x	adiponectin ~ treatment time ~ status
One-way ANOVA	Continuous	1 categorical (2 or more levels)	y~x	myoglobin ~ species
Two-way ANOVA	Continuous	2 categorical (2 or more levels each)	y~x1*x2	para ~ season * species diameter ~ agent * species

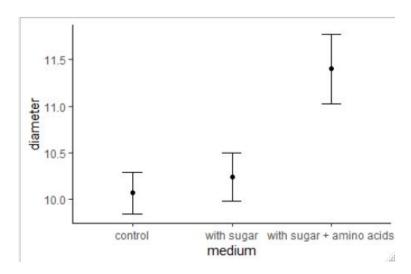
Why use Im()?

For example...

Procedure	Response	Explanatory	R	Stage 1 examples
Single linear regression	Continuous	1 Continuous	y~x	mand ~ jh mass ~ day
Two-sample t-test	Continuous	1 categorical (2 levels)	y~x	adiponectin ~ treatment time ~ status
One-way ANOVA	Continuous	1 categorical (2 or more levels)	y~x	myoglobin ~ species
Two-way ANOVA	Continuous	2 categorical (2 or more levels each)	y~x1*x2	para ~ season * species diameter ~ agent * species
	Continuous	1 categorical and 1 continuous	y~x1*x2	

Revisiting: One-way ANOVA

mod <- aov(y ~ x, data = mydata) summary(mod)



Revisiting: One-way ANOVA

```
modc <- aov(diameter ~ medium, data = culture)
summary(modc)

Df Sum Sq Mean Sq F value Pr(>F)
medium 2 10.495 5.2473 6.1129 0.00646 **
Residuals 27 23.177 0.8584

---
Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

Using Im()

```
modl <- lm(diameter ~ medium, data = culture)</pre>
summary(modl)
lm(formula = diameter ~ medium, data = culture)
Residuals:
  Min
          10 Median
                       30
-1.541 -0.700 -0.080 0.424 1.949
Coefficients:
                             Estimate Std. Error t value Pr(>|t|)
                              10.0700
                                          0.2930 34.370 < 2e-16 ***
(Intercept)
mediumwith sugar
                               0.1700
                                          0.4143 0.410 0.68483
mediumwith sugar + amino acids 1.3310
                                          0.4143
                                                 3.212 0.00339 **
Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
Residual standard error: 0.9265 on 27 degrees of freedom
Multiple R-squared: 0.3117, Adjusted R-squared: 0.2607
F-statistic: 6.113 on 2 and 27 DF, p-value: 0.00646
```

Revisiting: One-way ANOVA

Intercept is mean of 'lowest' level of factor

I.e., equivalent to x = 0 in regression

```
11.5
diameter
0.11
  10.5
  10.0
                                   with sugar with sugar + amino acids
                control
                                   medium
```

```
modl <- lm(diameter ~ medium, data = culture)</pre>
summary(mod1)
                                                   Control mean sig diff
lm(formula = diameter ~ medium, data = culture)
                                                   from 0. Not important
Residuals:
          10 Median
  Min
-1.541 -0.700 -0.080 0.424 1.949
Coefficients:
                              Estimate Std. Error t value Pr(>|t|)
(Intercept)
                               10,0700
                                           0.2930 34.370 < 2e-16 ***
mediumwith sugar
                                0.1700
                                           0.4143
                                                   0.410 0.68483
mediumwith sugar + amino acids
                               1.3310
                                           0.4143
                                                   3.212 0.00339 **
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```

Revisiting: One-way ANOVA

modl <- lm(diameter ~ medium, data = culture)</pre>

```
Difference between intercept and next level
```

```
11.5
diameter
0.11
  10.5
  10.0
                                   with sugar with sugar + amino acids
                control
                                   medium
```

```
summary(mod1)
lm(formula = diameter ~ medium, data = culture)
Residuals:
  Min
          10 Median
-1.541 -0.700 -0.080 0.424 1.949
Coefficients:
                              Estimate Std. Error t value Pr(>|t|)
(Intercept)
                              10.0700
                                          0.2930 34.370 < 2e-16 ***
mediumwith sugar
                               0.1700
                                          0.4143
                                                  0.410 0.68483
mediumwith sugar + amino acids
                               1.3310
                                          0.4143
                                                   3.212 0.00339 **
Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
Residual standard error: 0.9265 on 27 degrees of freedom
Multiple R-squared: 0.3117, Adjusted R-squared: 0.2607
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```

Revisiting: One-way ANOVA

modl <- lm(diameter ~ medium, data = culture)</pre>

Difference between intercept and third level

```
11.5
diameter
0.11
   10.5
  10.0
                                   with sugar with sugar + amino acids
                 control
                                   medium
```

```
summary(mod1)
lm(formula = diameter ~ medium, data = culture)
Residuals:
  Min
          10 Median
-1.541 -0.700 -0.080 0.424 1.949
Coefficients:
                              Estimate Std. Error t value Pr(>|t|)
(Intercept)
                              10.0700
                                          0.2930 34.370 < 2e-16 ***
mediumwith sugar
                               0.1700
                                          0.4143
                                                  0.410 0.68483
mediumwith sugar + amino acids
                              1.3310
                                          0.4143
                                                  3.212 0.00339 **
Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
Residual standard error: 0.9265 on 27 degrees of freedom
Multiple R-squared: 0.3117, Adjusted R-squared: 0.2607
F-statistic: 6.113 on 2 and 27 DF, p-value: 0.00646
```

Usual steps in applying Im()

```
lm()
```

summary(mod1) - 'estimates' and direction of effects

- +'ve bigger than intercept
- -'ve smaller than intercept

```
modl <- lm(diameter ~ medium, data = culture)</pre>
summary(mod1)
lm(formula = diameter ~ medium, data = culture)
Residuals:
  Min
          10 Median
                              Max
-1.541 -0.700 -0.080 0.424 1.949
Coefficients:
                              Estimate Std. Error t value Pr(>|t|)
                               10.0700
(Intercept)
                                           0.2930 34.370 < 2e-16 ***
                                0.1700
mediumwith sugar
                                           0.4143
                                                    0.410
                                                          0.68483
mediumwith sugar + amino acids 1.3310
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                                                    3.212 0.00339 **
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 0.9265 on 27 degrees of freedom
Multiple R-squared: 0.3117, Adjusted R-squared: 0.2607
F-statistic: 6.113 on 2 and 27 DF, p-value: 0.00646
```

Usual steps in applying Im()

anova(mod1)

Test of the 'explanatory power' of the model

For reference: it's also how to compare models

Usual steps in applying Im()

Post hoc - which means differ

Use glht() from package multcomp

Torsten Hothorn, Frank Bretz and Peter Westfall (2008), Simultaneous Inference in General Parametric Models. *Biometrical Journal*, **50**(3), 346--363

```
library(multcomp)
post <- glht(mod1, linfct = mcp(medium = "Tukev"))</pre>
summary(post)
       Simultaneous Tests for General Linear Hypotheses
Multiple Comparisons of Means: Tukey Contrasts
Fit: lm(formula = diameter ~ medium, data = culture)
Linear Hypotheses:
                                           Estimate Std. Error t value Pr(>|t|)
                                                        0.4143
with sugar - control == 0
                                             0.1700
                                                                        0.91168
with sugar + amino acids - control == 0 1.3310
                                                       0.4143
                                                                 3.212
                                                                       0.00912 **
with sugar + amino acids - with sugar == 0 1.1610
                                                       0.4143
                                                                 2.802
                                                                       0.02442 *
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)
```

Assumptions - exactly as stage 1

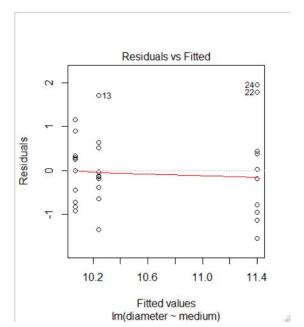
```
shapiro.test(mod1$residuals)
```

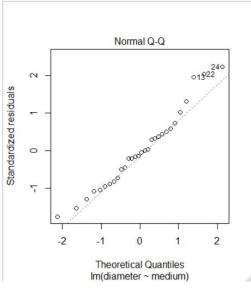
Shapiro-Wilk normality test

data: mod1\$residuals
W = 0.96423, p-value = 0.3953

plot(mod1)

These look fine





Key points

T-tests, ANOVA and regression are fundamentally the same, collectively called 'general linear models'. They can be carried out in R with Im()

The concept can be extended to 'generalised linear models' for different types of response. Generalised linear models are carried out in R with glm()

The output of Im() looks more complex, at first, than the outputs of t.test() and aov()

The output of glm() is like that for lm(). So we will revisit regression, t-tests and ANOVA using lm() to help you understand the output