Exercises for Introduction to Linear Models: ANOVAs, Multiple Regression and ANCOVA

Suggested Solutions

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Exercise 1: Clinical trials

A clinical trial was conducted to study the effect of the levels of a drug on some measure of well-being (called `SCORE` in this case). Both male and female subjects were randomly choosen for the study and then randomly assigned to to either a low or a high `DOSE` treatment.

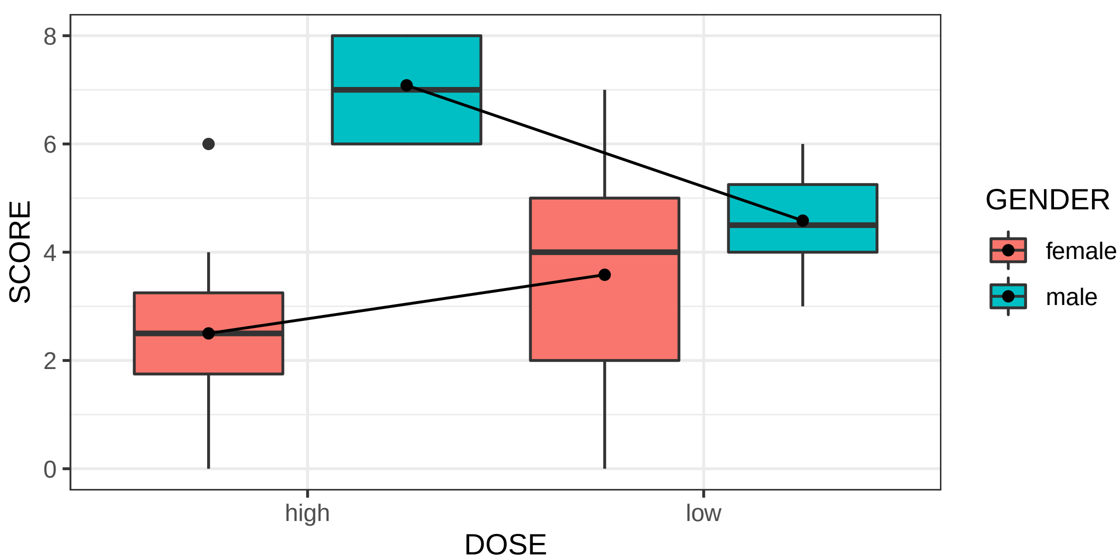
To read this data, directly from the web, into an object called `drugTrials`, do the following in R:

```
drugTrials <- read.csv("https://git.io/JeoOu")
```

Make sure the data is read in correctly. If it is read correctly, there should be 48 rows and 4 columns (we don't need the first column which is unique ID for each individual).

1.) Perform some exploratory analysis on the data set and answer the following questions: (a) Do you think the SCOREs are different between genders? (b) are SCOREs different between differend doses of the drug? (c) is there an interaction between GENDER and DOSE? (d) is this data set balanced (i.e. equal observations across all subgroups)?

```
# make a boxplot for all 4 subgroups
require(ggplot2)
p <- ggplot(drugTrials, aes(x = DOSE, y = SCORE, fill = GENDER))
p + geom_boxplot(position = position_dodge(1)) + stat_summary(fun.y = mean, geom = "line",
    aes(group = GENDER), position = position_dodge(1)) + stat_summary(fun.y = mean,
    geom = "point", position = position_dodge(1)) + theme_bw()
```



It is difficult to discern any main effects here - they all seem to be context dependent which suggests there is an interaction between DOSE and GENDER in determing the SCORE is the dataset balanced?

```r
table(drugTrials$GENDER, drugTrials$DOSE)
```

```
##
##          high low
##   female   12  12
##   male     12  12
```

Yes it is, equal number of observations for each subgroup, which means when you can the `aov()` function to generate the 2-way ANOVA table

2.) Carry out a two-way ANOVA with interaction on this data set and answer the following questions (a) Is the interaction effect signficant? (b) are any of the main effects signficant? (c) if the interaction is signficant can you still easily interpret the results for any significant main effects?

```r
drug_anova <- aov(SCORE ~ GENDER * DOSE, data = drugTrials)
summary(drug_anova)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## GENDER       1  93.52   93.52  37.494 2.22e-07 ***
## DOSE         1   6.02    6.02   2.414 0.127433
## GENDER:DOSE  1  38.52   38.52  15.443 0.000297 ***
## Residuals   44 109.75    2.49
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Always start from the bottom (the interaction term), which is signficant (F_1,44 = 15.4, p-value= 0.0003). The main effect of gender is also signicant, but it is not possible to say that Males are different from Females for SCORES, because of the significance of the interaction term (just look the boxplot again)

3.) Carry out the appropriate Tukey Post-hoc tests and examine the results. do they contradict your interpretation of 2(c) ?

```r
# again because of the signficant interactions we need to look at pairwise
# comparisons of the interaction term i.e. all the subgroups against one another
TukeyHSD(drug_anova, which = "GENDER:DOSE")
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = SCORE ~ GENDER * DOSE, data = drugTrials)
##
## $`GENDER:DOSE`
##                             diff        lwr        upr     p adj
## male:high-female:high   4.583333  2.8618117  6.3048550 0.0000000
## female:low-female:high  1.083333 -0.6381883  2.8048550 0.3461255
## male:low-female:high    2.083333  0.3618117  3.8048550 0.0120658
## female:low-male:high   -3.500000 -5.2215216 -1.7784784 0.0000135
## male:low-male:high     -2.500000 -4.2215216 -0.7784784 0.0019117
## male:low-female:low     1.000000 -0.7215216  2.7215216 0.4166923
```

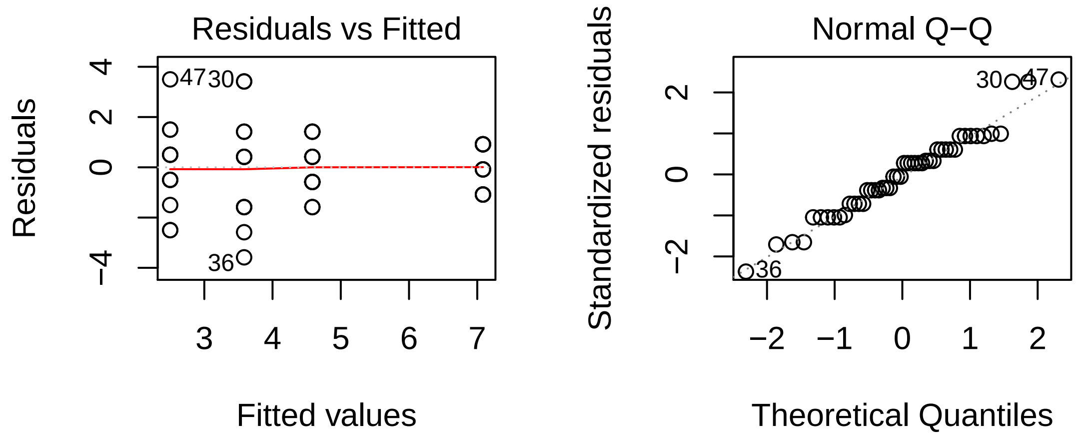
Note the genders are not significantly different when both receive a low dose of the Drug but the difference is quite pronounced when both recieve a high dose. Females don't seem to be responsive to differences in the level of drug, whereas males *do* seem to respond to different levels of the drug.

4.) Evaluate the assumptions of the anova model for the drug trial data? Are there any assumptions violated? Which one might be the most problematic?

```r
# draw multiple base plots in same window
par(mfrow = c(1, 2))

# assumption 2: homogeneity of variance
plot(drug_anova, 1)
# There does seem to be more spread on the left hand side but its not too extreme

# assumption 3 of normality of residuals
plot(drug_anova, 2)
```




```r
# the 2nd plot looks a bit weird and maybe the worst of the lot but remember
# ANOVAs are robust to small deviations from normality
```

Exercise 2: The Diet Experiment

This data for this study comes from an experiment in which people were put on one of three diets to encourage weight gain. Additionally the diets were trialled independently in three different countries.

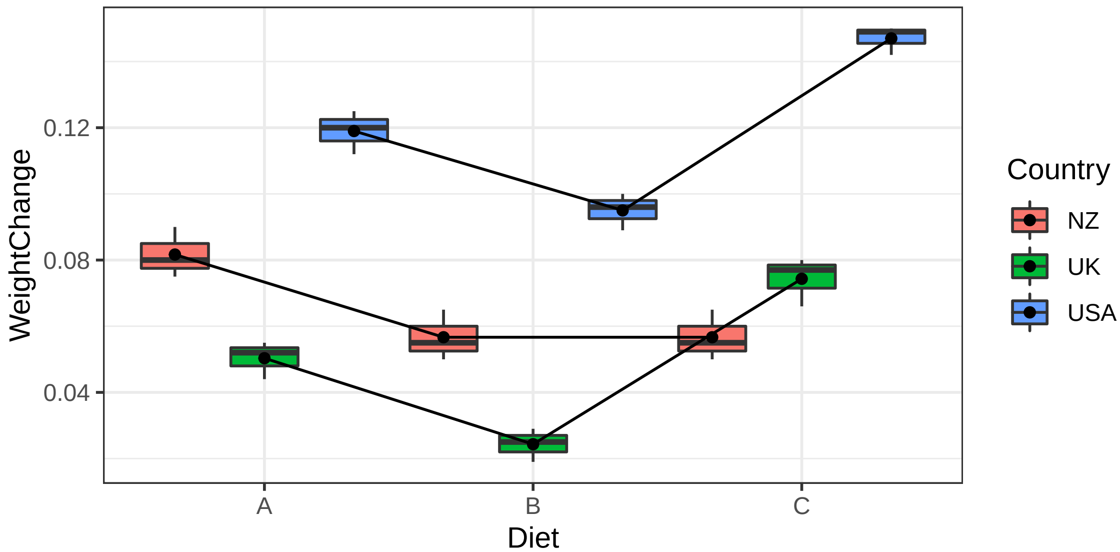
To read this data, directly from the web, into an object called `dietData`, do the following in R:

```r
dietData <- read.csv("https://git.io/Jeo04")
```

Make sure the data is read in correctly. If it is read correctly, there should be 27 rows and 3 columns.

1.) Perform some exploratory analysis on the data set and answer the following questions: (a) Do you think the different diets are equally effective for weight gain? (b) How do you think country of origin might influence weight gain? (d) is this data set balanced (i.e. equal observations across all subgroups)?

```r
# make a boxplot for all 9 subgroups
require(ggplot2)
p <- ggplot(dietData, aes(x = Diet, y = WeightChange, fill = Country))
p <- p + geom_boxplot(position = position_dodge(1))
p <- p + stat_summary(fun.y = mean, geom = "line", aes(group = Country), position = position_dodge(1))
p <- p + stat_summary(fun.y = mean, geom = "point", position = position_dodge(1))
p + theme_bw()
```



It is easy to see that individuals from the US had the highest change in weight regardless of diet. However it is difficult to guess which diet is most effective. There migh

is the dataset balanced?

```
table(dietData$Diet, dietData$Country)
```

```
##
##     NZ UK USA
##   A  3  3   3
##   B  3  3   3
##   C  3  3   3
```

Yes it is, equal number of observations for each subgroup, which means when you can the `aov()` function to generate the 2-way ANOVA table

2.) Carry out a two way ANOVA with interaction and Tukey post-hoc tests to determine which diet is most effective for weight gain? Is there a single answer to the previous question? Why or why not? Note a visualization with CI's may be an easier way to intepret the results.

```
diet_anova <- aov(WeightChange ~ Country * Diet, data = dietData)
summary(diet_anova)
```

```
##              Df   Sum Sq  Mean Sq F value   Pr(>F)
## Country       2 0.024872 0.012436  294.54 1.77e-14 ***
## Diet          2 0.005586 0.002793   66.15 5.07e-09 ***
## Country:Diet  4 0.003480 0.000870   20.61 1.60e-06 ***
## Residuals    18 0.000760 0.000042
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The post-hoc tests for only the interaction term again:

```
TukeyHSD(diet_anova, "Country:Diet")
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = WeightChange ~ Country * Diet, data = dietData)
##
```

```
## $`Country:Diet`
##                   diff          lwr          upr     p adj
## UK:A-NZ:A   -0.031333333 -0.0499230134 -0.012743653 0.0003699
## USA:A-NZ:A   0.037333333  0.0187436533  0.055923013 0.0000412
## NZ:B-NZ:A   -0.025000000 -0.0435896800 -0.006410320 0.0042561
## UK:B-NZ:A   -0.057333333 -0.0759230134 -0.038743653 0.0000001
## USA:B-NZ:A   0.013333333 -0.0052563467  0.031923013 0.2881070
## NZ:C-NZ:A   -0.025000000 -0.0435896800 -0.006410320 0.0042561
## UK:C-NZ:A   -0.007333333 -0.0259230134  0.011256347 0.8905739
## USA:C-NZ:A   0.065333333  0.0467436533  0.083923013 0.0000000
## USA:A-UK:A   0.068666667  0.0500769866  0.087256347 0.0000000
## NZ:B-UK:A    0.006333333 -0.0122563467  0.024923013 0.9477970
## UK:B-UK:A   -0.026000000 -0.0445896800 -0.007410320 0.0028799
## USA:B-UK:A   0.044666667  0.0260769866  0.063256347 0.0000035
## NZ:C-UK:A    0.006333333 -0.0122563467  0.024923013 0.9477970
## UK:C-UK:A    0.024000000  0.0054103200  0.042589680 0.0062915
## USA:C-UK:A   0.096666667  0.0780769866  0.115256347 0.0000000
## NZ:B-USA:A  -0.062333333 -0.0809230134 -0.043743653 0.0000000
## UK:B-USA:A  -0.094666667 -0.1132563467 -0.076076987 0.0000000
## USA:B-USA:A -0.024000000 -0.0425896800 -0.005410320 0.0062915
## NZ:C-USA:A  -0.062333333 -0.0809230134 -0.043743653 0.0000000
## UK:C-USA:A  -0.044666667 -0.0632563467 -0.026076987 0.0000035
## USA:C-USA:A  0.028000000  0.0094103200  0.046589680 0.0013235
## UK:B-NZ:B   -0.032333333 -0.0509230134 -0.013743653 0.0002541
## USA:B-NZ:B   0.038333333  0.0197436533  0.056923013 0.0000290
## NZ:C-NZ:B    0.000000000 -0.0185896800  0.018589680 1.0000000
## UK:C-NZ:B    0.017666667 -0.0009230134  0.036256347 0.0699594
## USA:C-NZ:B   0.090333333  0.0717436533  0.108923013 0.0000000
## USA:B-UK:B   0.070666667  0.0520769866  0.089256347 0.0000000
## NZ:C-UK:B    0.032333333  0.0137436533  0.050923013 0.0002541
## UK:C-UK:B    0.050000000  0.0314103200  0.068589680 0.0000007
## USA:C-UK:B   0.122666667  0.1040769866  0.141256347 0.0000000
## NZ:C-USA:B  -0.038333333 -0.0569230134 -0.019743653 0.0000290
## UK:C-USA:B  -0.020666667 -0.0392563467 -0.002076987 0.0229027
## USA:C-USA:B  0.052000000  0.0334103200  0.070589680 0.0000004
## UK:C-NZ:C    0.017666667 -0.0009230134  0.036256347 0.0699594
## USA:C-NZ:C   0.090333333  0.0717436533  0.108923013 0.0000000
## USA:C-UK:C   0.072666667  0.0540769866  0.091256347 0.0000000
```

```r
# A lot of results
```

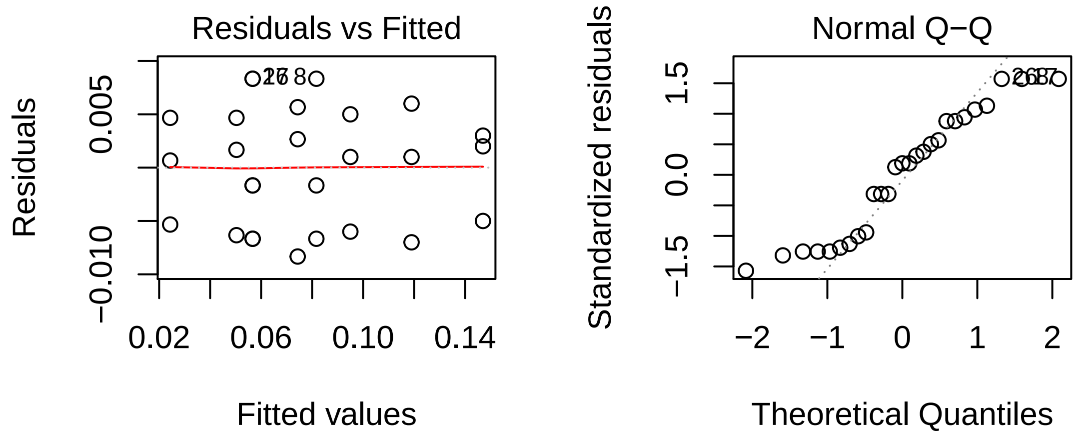
There is a lot to summarize here. Plotting the interaction plot with 95% Confidence intervals might be more instructive, then sifting through this table.

3.) Evaluate the assumptions of the anova model for the drug trial data. Are there any assumptions violated? Which one might be the most problematic?

```r
# draw multiple base plots in same window
par(mfrow = c(1, 2))

# assumption 2: homogeneity of variance
plot(diet_anova, 1)
# This looks OK.

# assumption 3 of normality of residuals
plot(diet_anova, 2)
```

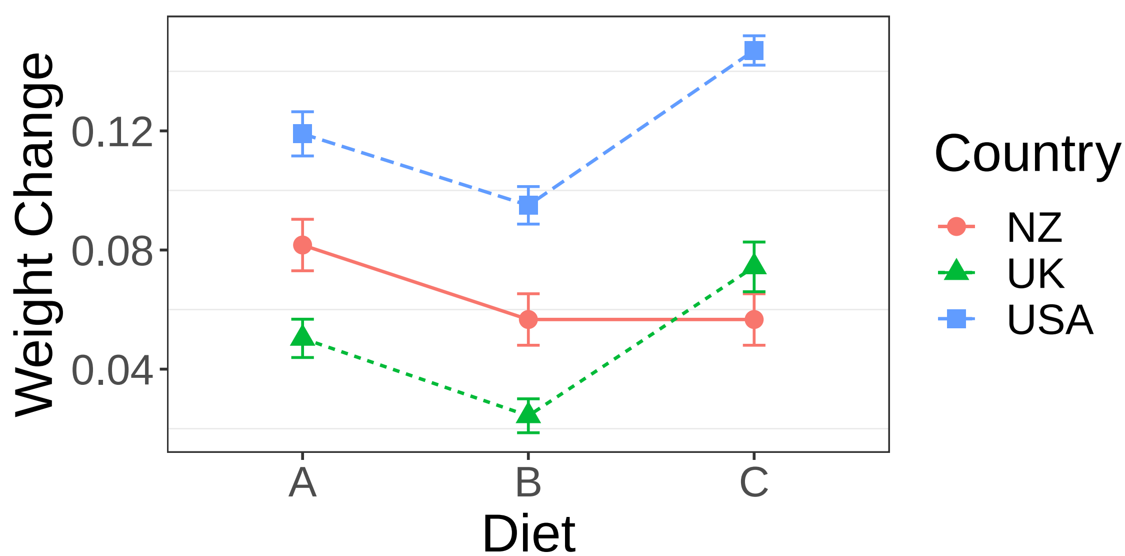



```
# again the 2nd plot looks a bit weird and maybe the worst of the lot but
# remember ANOVAs are robust to small deviations from normality
```

4.) Visualize your results as interaction plot (as in the lecture slides), make sure to provide 95% CI for means of all subgroups.

```
# Summarize the data in a new data frame
df <- with(dietData, aggregate(WeightChange, list(Diet = Diet, Country = Country),
    mean))
# note: the standard error of the mean is 1 sd of the mean, we need this to get
# the 95% CI
df$se <- with(dietData, aggregate(WeightChange, list(Diet = Diet, Country = Country),
    function(x) sd(x)/sqrt(3)))[, 3]

gp <- ggplot(df, aes(x = Diet, y = x, colour = Country, group = Country))
gp <- gp + geom_line(aes(linetype = Country), size = 0.6)
gp <- gp + geom_point(aes(shape = Country), size = 3)
gp <- gp + geom_errorbar(aes(ymax = x + (1.96 * se), ymin = x - (1.96 * se)), width = 0.1)
gp <- gp + scale_x_discrete(name = "Diet", labels = c("A", "B", "C"))
gp <- gp + scale_y_continuous(name = "Weight Change")
gp <- gp + theme_bw()
gp + theme(panel.grid.major = element_blank(), text = element_text(size = 20))
```



Overall Diet A seems to be better than diet B. However for Americans and Britons, diet C seems to the most effective. Diets B and C are equally effective if you happen to be a New Zealander. As long as the 95% CI bars don't overlap, we know two means will be signficantly different (at the alpha=0.05 level). This is not true if just plotted standard errors (which are 68% CIs).

Exercise 3: Swiss Fertility data

For the follow exercise we will use a built-in data set in R. To load the dataset, type the following in R

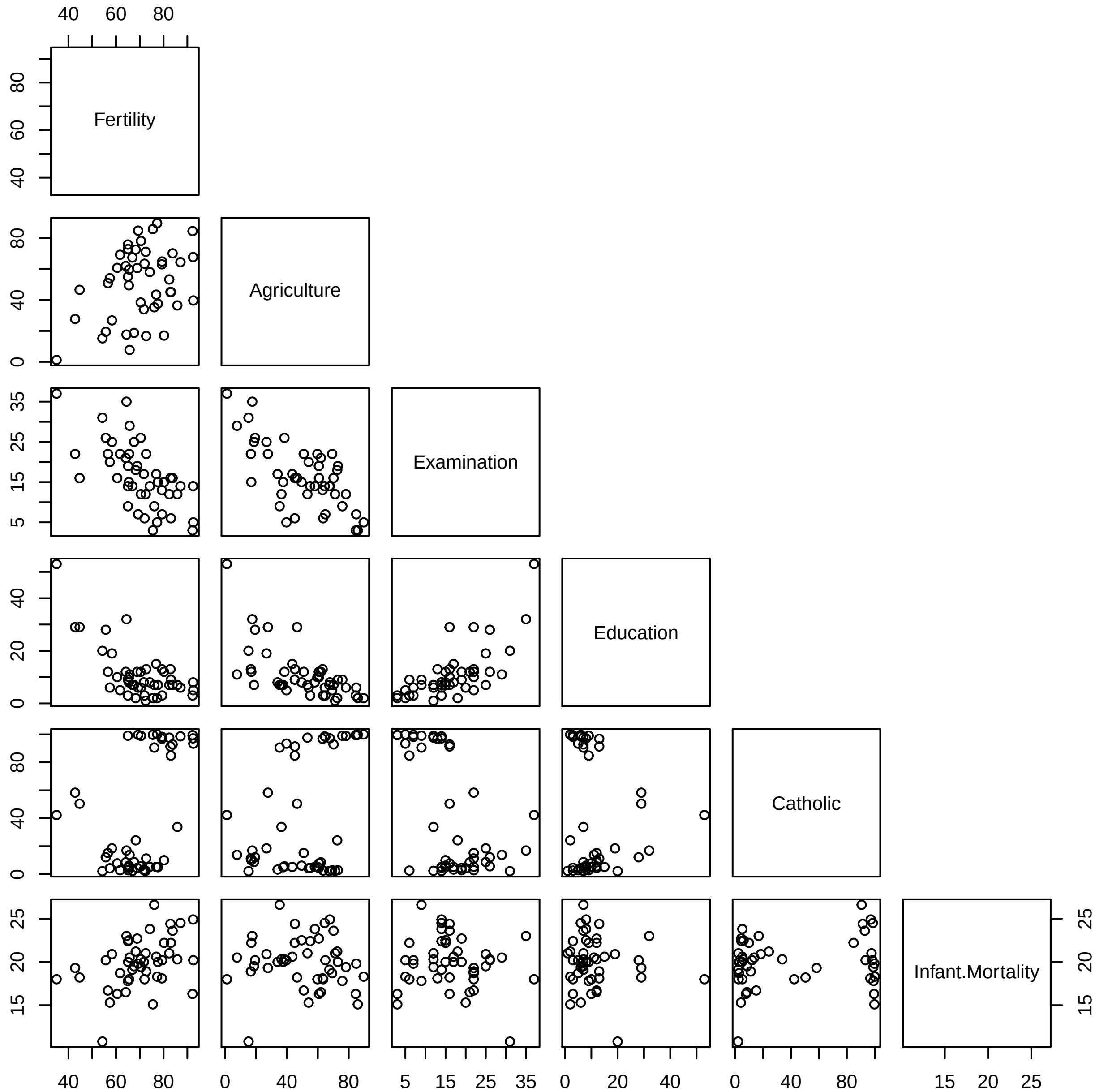
```r
data(swiss)
```

Briefly this data includes a standardized fertility measure from 47 french-speaking provinces in Switzerland from 1888. It also includes 5 socio-economic indicators of the provinces as well. For further details type `help(swiss)`. All variables have been scaled to a numerical continuous scale.

We would like to understand what influence, if any, the 5 socio-economic factors have on fertility.

1. Use the `pairs()` function to draw scatterplots of all 6 variables with one another: (a) Which variables seem most correlated with `Fertility` (b) Which variables seem highly correlated with one another? (c) Which variable would you choose to model changes in fertility?

```r
pairs(swiss, upper.panel = NULL)
```



Note all 5 variables seem correlated with fertility. The correlation between fertility and catholic does not seem very linear. Additionally, the variables examination, education and agriculture seem correlated with one another. As a first attempt we could fit all 5 variables as explanatory variables for fertility keeping in mind the multicollinearity of the 3 variables and non-linear relationship between fertility and catholic.

2. Use the following code to fit all 5 variables as the explanatory variables for `fertility` and the save the fit in '`fit1`:

```
# this will use all variables other than fertility as explanatory variables in a
# linear model
fit1 <- lm(Fertility ~ ., data = swiss)
```

Call `summary()` on the saved model. Are all the slopes/coefficients signficant? Are there any results surprsing based on your plots from (1) above?

```
summary(fit1)
```

```
##
## Call:
## lm(formula = Fertility ~ ., data = swiss)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -15.2743  -5.2617   0.5032   4.1198  15.3213
##
## Coefficients:
##                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)      66.91518   10.70604   6.250 1.91e-07 ***
## Agriculture      -0.17211    0.07030  -2.448  0.01873 *
## Examination      -0.25801    0.25388  -1.016  0.31546
## Education        -0.87094    0.18303  -4.758 2.43e-05 ***
## Catholic          0.10412    0.03526   2.953  0.00519 **
## Infant.Mortality  1.07705    0.38172   2.822  0.00734 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 7.165 on 41 degrees of freedom
## Multiple R-squared:  0.7067, Adjusted R-squared:  0.671
## F-statistic: 19.76 on 5 and 41 DF,  p-value: 5.594e-10
```

All coefficients are signifcant (<0.05) other than examination. We clearly saw examination having a relationshio with ferility in the plots above. What could be the cause of this? Additionally, agriculture seems positively correlated with negative fertility, so why does it have a negative coefficent (it is signficant)?

3. Use the `vif()` function from the `car` package, on `fit1` to find the variable that leads to the most variance inflation. Fit a new linear model that omits this variable but retains the other 4 variables, save this model as `fit2`. Perform a nested likelihood ratio to test which model `fit1` or `fit2` is more appropriate. **Note** if the `car` package is not installed, you will have to install it yourself.

```r
library(car)
```

```
## Warning: package 'car' was built under R version 3.4.3
```

```r
vif(fit1)
```

```
##      Agriculture      Examination        Education         Catholic
##         2.284129         3.675420         2.774943         1.937160
## Infant.Mortality
##         1.107542
```

```r
# Examination has the highest vif so lets drop this from the model the following
# is a quicker way to do so
fit2 <- update(fit1, . ~ . - Examination)
# verify this does what we want it to do
summary(fit2)
```

```
##
## Call:
## lm(formula = Fertility ~ Agriculture + Education + Catholic +
##     Infant.Mortality, data = swiss)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -14.6765  -6.0522   0.7514   3.1664  16.1422
```

```
##
## Coefficients:
##                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)      62.10131    9.60489   6.466 8.49e-08 ***
## Agriculture      -0.15462    0.06819  -2.267  0.02857 *
## Education        -0.98026    0.14814  -6.617 5.14e-08 ***
## Catholic          0.12467    0.02889   4.315 9.50e-05 ***
## Infant.Mortality  1.07844    0.38187   2.824  0.00722 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 7.168 on 42 degrees of freedom
## Multiple R-squared:  0.6993, Adjusted R-squared:  0.6707
## F-statistic: 24.42 on 4 and 42 DF,  p-value: 1.717e-10
```

```r
# perform the likelihood ratio test
anova(fit1, fit2)
```

```
## Analysis of Variance Table
##
## Model 1: Fertility ~ Agriculture + Examination + Education + Catholic +
##     Infant.Mortality
## Model 2: Fertility ~ Agriculture + Education + Catholic + Infant.Mortality
##   Res.Df    RSS Df Sum of Sq      F Pr(>F)
## 1     41 2105.0
## 2     42 2158.1 -1   -53.027 1.0328 0.3155
```

Dropping the Examination term has negligble effect on the model.

4. Repeat the process above to find the the variable that leads to the most variance inflation in `fit2`. Fit a new linear model that omits this variable but retains the other 3 variables, save this model as `fit3`. Perform a nested likelihood ratio to test which model `fit1`, `fit2` or `fit3` is more appropriate.

```r
vif(fit2)
```

```
##      Agriculture        Education         Catholic Infant.Mortality
##         2.147153         1.816361         1.299916         1.107528
```

```r
# This time agriculture has the highest vif this isn't that hight but let's
# remove it anyway
fit3 <- update(fit2, . ~ . - Agriculture)
# verify this does what we want it to do
summary(fit3)
```

```
##
## Call:
## lm(formula = Fertility ~ Education + Catholic + Infant.Mortality,
##     data = swiss)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -14.4781  -5.4403  -0.5143   4.1568  15.1187
##
## Coefficients:
##                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)      48.67707    7.91908   6.147 2.24e-07 ***
## Education        -0.75925    0.11680  -6.501 6.83e-08 ***
```

```
## Catholic          0.09607    0.02722   3.530  0.00101 **
## Infant.Mortality  1.29615    0.38699   3.349  0.00169 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 7.505 on 43 degrees of freedom
## Multiple R-squared:  0.6625, Adjusted R-squared:  0.639
## F-statistic: 28.14 on 3 and 43 DF,  p-value: 3.15e-10
```

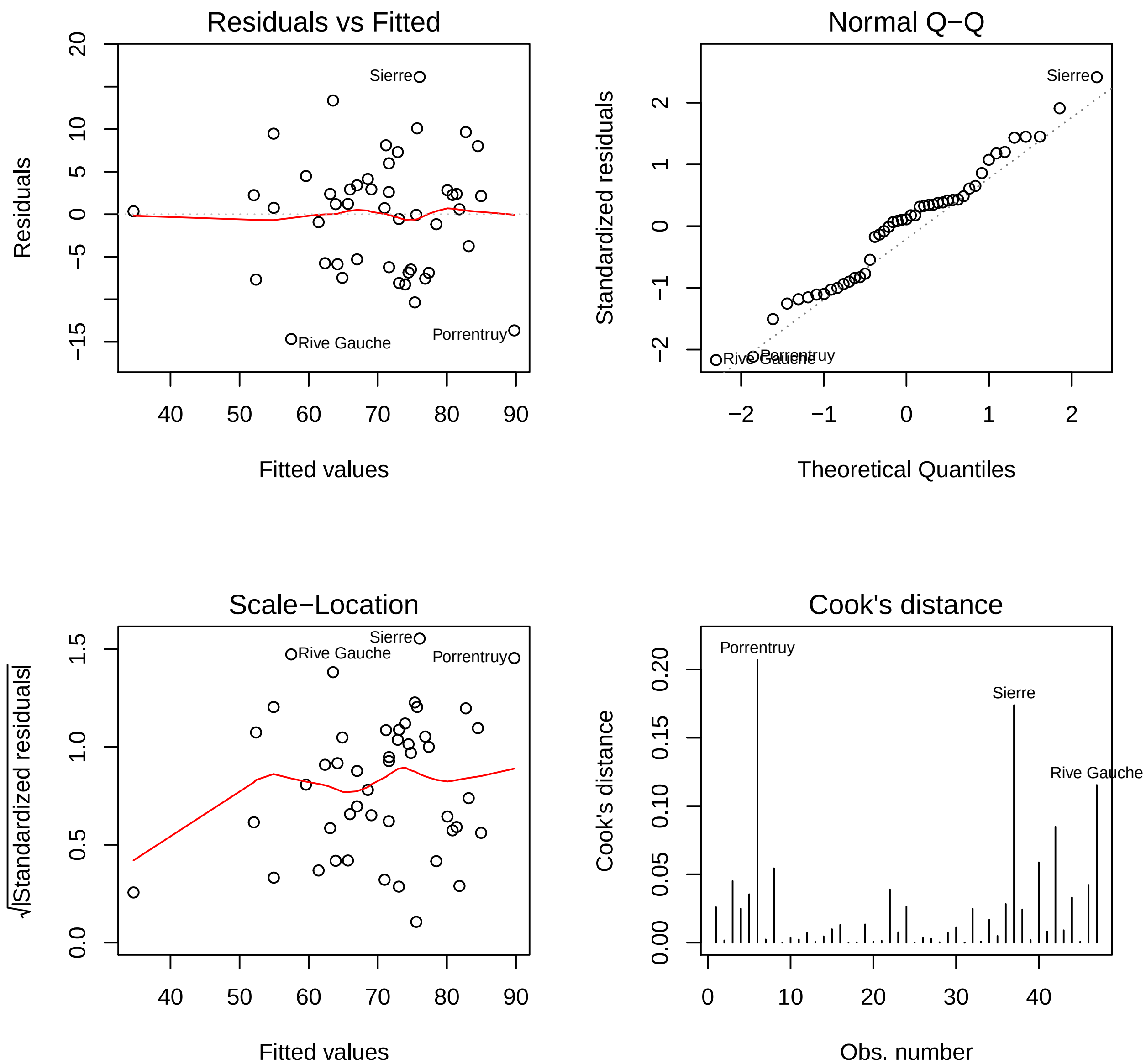
```r
# perform the likelihood ratio test
anova(fit1, fit2, fit3)
```

```
## Analysis of Variance Table
##
## Model 1: Fertility ~ Agriculture + Examination + Education + Catholic +
##     Infant.Mortality
## Model 2: Fertility ~ Agriculture + Education + Catholic + Infant.Mortality
## Model 3: Fertility ~ Education + Catholic + Infant.Mortality
##   Res.Df    RSS Df Sum of Sq      F  Pr(>F)
## 1     41 2105.0
## 2     42 2158.1 -1   -53.027 1.0328 0.31546
## 3     43 2422.2 -1  -264.176 5.1454 0.02864 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Dropping agriculture does has have a signficant effect and we are better off keeping this in the model

5. For the best fitting model from (4) check all the assumptions of the multiple linear regression model. Do any of them seem particularly problematic?

```r
par(mfrow = c(2, 2))
plot(fit2, 1)
plot(fit2, 2)
plot(fit2, 3)
plot(fit2, 4)
```



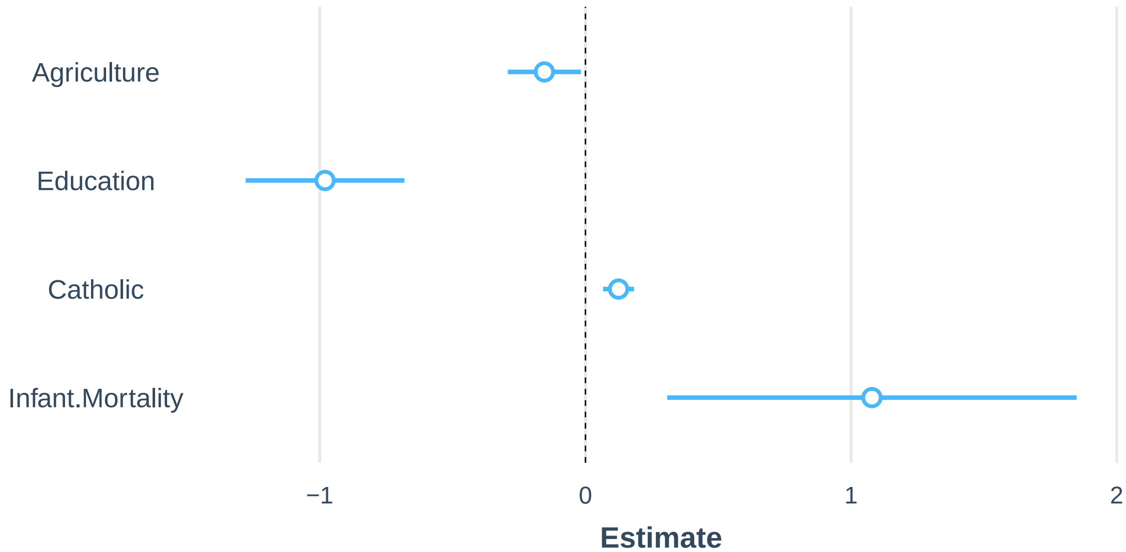
The biggest problem might that there is an outlier or two otherwise this not too bad.

6. Plot the model coeffecients with 95% confidence intervals to visualize which variables appear to have the largest effect on fertility?

You could use the strategy from exercise 2 above or the following shortcut which requires you install the package “jtools”

```
# You might want to uncomment the line below and install jtools
# install.packages('jtools')

# once that is done, load the package to use the functions within it
library(jtools)
# the plotting is as easy as..
plot_coefs(fit2)
```



Notice education and infant mortalty seem to have the largest magnitude of effect on fertility, negative for education and positive for infant mortality. Aren't plots much simpler to interpret than a bunch of numbers??

Exercise 4: Stickleback Association Study

The dataset for this exercise consists of 1682 single nucleotide polymorphism (SNP) markers, typed across 396 adult threespine stickleback fish (*Gasterosteus aculeatus*). There is an enormous range of morphological variation present within three-spined sticklebacks. These fall into two main categories, the **anadromous** and the **freshwater** forms. One major difference between the two categories of forms is the number of armour plates along the lateral side of the fish. Anadromous fish are heavily plated and have upwards of 25 plates on each side, whereas freshwater fish only have about 5 on each side (see the image below)

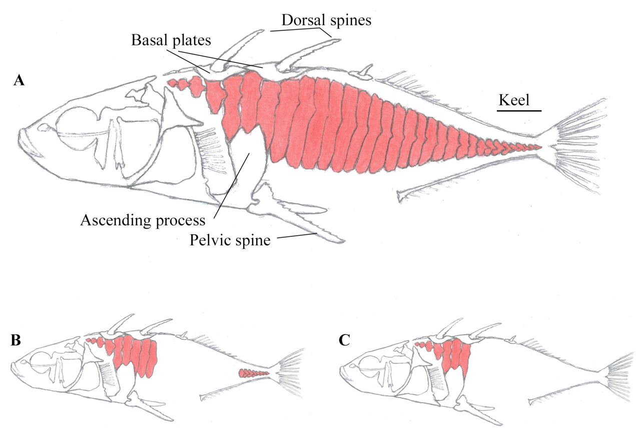


Fig Source: Wiig E, Reseland JE, Østbye K, Haugen HJ, Vøllestad LA (2016) Variation in Lateral Plate Quality in Threespine Stickleback from Fresh, Brackish and Marine Water: A Micro-Computed Tomography Study. PLoS ONE 11(10): e0164578. https://doi.org/10.1371/journal.pone.0164578

The data for the 328 fish comes from adults caught in an **admixture zone**. In the admixture zone, anadramous and freshwater types meet and breed freely, allowing for recombination and linkage disequilibrium between genetic markers and phenotypes. Hence, these *hybrid* individuals are ideal for identifying the genetic basis of traits that differ in the parental popualtions (anadromous *vs.* freshwater). **We will use this dataset to find the genetic loci associated with the number of armour plates**

Read in the dataset as follows (this make take some time):

```
stickle <- read.csv("https://git.io/Jer8h")
```

Make sure the data set has 328 rows (adult fish) and 1686 columns. Here is a brief description of the variables:

The first column `ind` is a unique identifier for each fish. You likely will not need this for anything.

Columns 2:1683 are all the genetic marker. The name of each genetic marker starts with the chromosome number followed by a . then the marker position in bases followed by some additional information that is not relevant here.

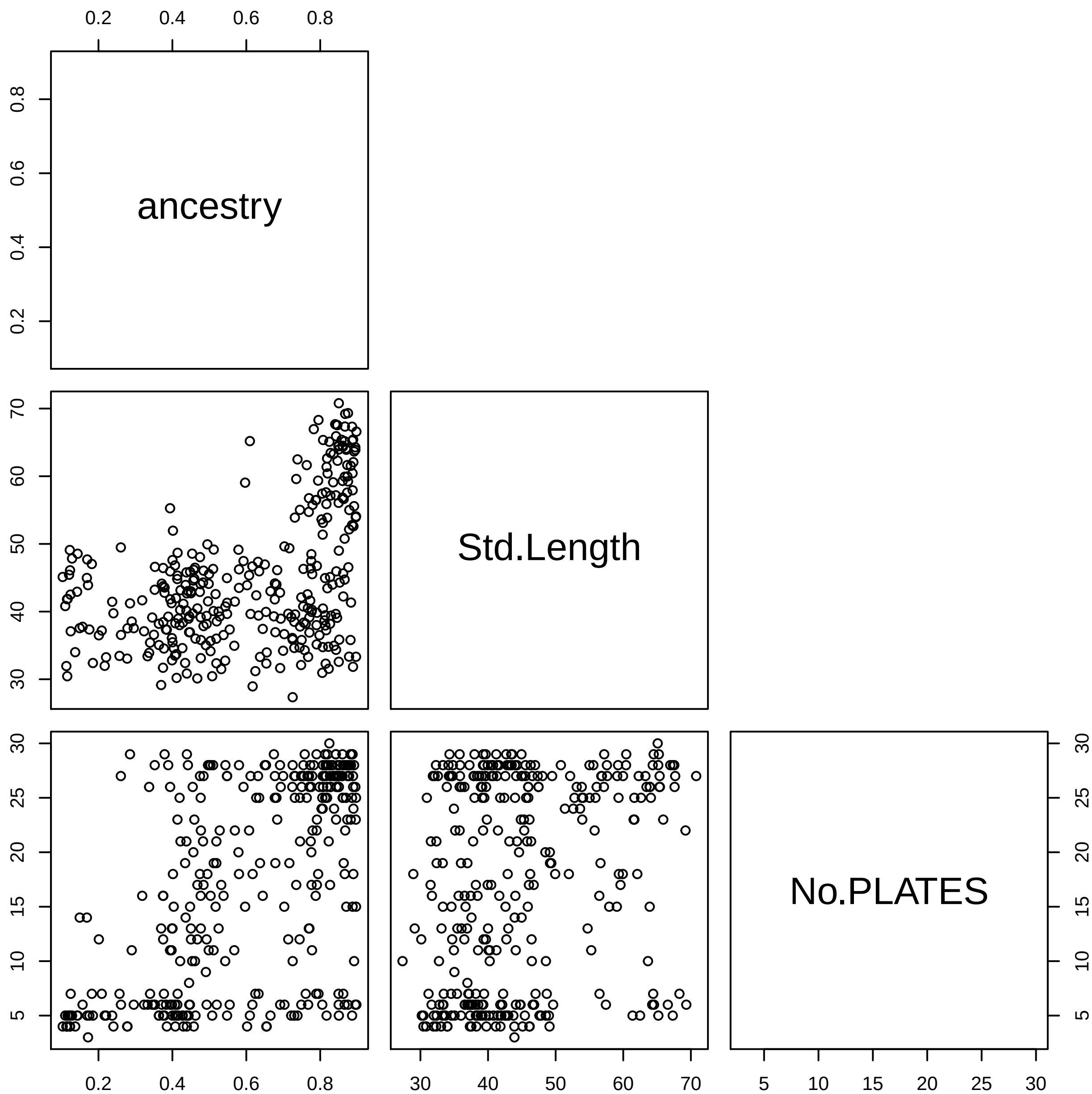
`Ancestry` is the predicted ancestry for each individual fish. This number ranges from 0-1. A number closer to zero means the fish has mostly a freshwater genetic makeup, while a number close to 1 means the fish has mostly an anadromous genetic makeup.

`Std.Length`is a numerical variable the represents the size of the fish (in cm)

`No.PLATES` is the number of armour plates along the lateral side of the fish

1.) Explore the relationship between Number of plates and the other numerical variables (Ancestry and Std. length). Would you expect a relationship between any of these variables? What does the data suggest?

```
# plotting only the numerical variables against one another
pairs(stickle[1684:1686], upper.panel = NULL)
```



Note ancestry seems to be weakly correlated with both Number of plates and Std. Length. However, there is not much going on between Std.Length and Number of plates.

2.) Based on your exploratory analysis above, which, if any of Std.length or Ancestry seem correlated with No.Plates? Fit a linear model with one or both of these as explanatory variables and No.Plates as the response variable and call it `baseline`. How much variance in No.Plates does your model explain (Adjusted R Squared)?

```r
baseline <- lm(No.PLATES ~ ancestry, data = stickle)
summary(baseline)
```

```
##
## Call:
## lm(formula = No.PLATES ~ ancestry, data = stickle)
##
## Residuals:
```

```
##     Min      1Q  Median      3Q     Max
## -18.893  -5.393   1.121   5.125  19.267
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)    3.010      1.185   2.539   0.0116 *
## ancestry      23.565      1.836  12.836   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 7.646 on 326 degrees of freedom
## Multiple R-squared:  0.3357, Adjusted R-squared:  0.3337
## F-statistic: 164.8 on 1 and 326 DF,  p-value: < 2.2e-16
```

Ancestry explains about 33% of the variance in the number of plates. At this point it might be worth checking model assumptions as well. Most of the model assumptions are met but there might be some heterogeneity of variances, but it's not extreme. A transformation may improve the model but we'll continue as is.

3.) Iteratively add each snp marker to the `baseline` above to create another model. Compare the two models using the likeleihood ratio test from the `anova` function. Make sure you do this for each of the 1682 markers and store the p-value of the likelihood ratio test in another dataframe along with the name of the marker. After doing this for all markers, use the bonferroni correction ($\frac{\alpha}{\text{no. markers}}$) to find all markers with signficant association with the number of plates.

```r
# get names of each marker
markernames <- names(stickle)[2:1683]

# make a dataframe to store the results
ptable <- data.frame(marker = markernames, pval = numeric(length = 1682))

# loop through each marker
for (i in seq_along(markernames)) {
    # note some markers are monomorphic, it would be better to remove them first,
    # they will generate warnings if you don't remove them

    # make a model of No.PLATES ~ ancestry + markername
    marker_model <- lm(reformulate(c("ancestry", markernames[i]), "No.PLATES"), data = stickle)
    # get the pvalue from the likelihood ratio test of the baseline model vs the
    # baseline + marker model
    ptable$pval[i] <- anova(baseline, marker_model)$`Pr(>F)`[2]
}

# the bonferonni alpha value calculation
bonferroni_alpha = 0.05/length(markernames)

significant_markers <- ptable[ptable$pval < bonferroni_alpha, ]
significant_markers
```

```
##                               marker         pval
## 112             chrIV.12814920_Ht32_1 1.998876e-37
## 172       chrIV.11993728_DSL21_88.1_0 6.446328e-09
## 210       chrIV.12864733_DSL21_96.1_0 3.492607e-16
## 239              chrI.21825411_Hp23_1 5.941047e-06
## 272             chrIV.12831803_Hp47_1 6.700604e-32
## 544             chrIV.12815271_Hp46_1 3.410510e-34
## 624       chrIV.12801903_DSL21_95.3_0 1.148764e-42
```

```
## 625                   chrIV.12817401_Hp45_1 1.308608e-59
## 638             chrIV.11994084_DSL21_88.2_0 3.719141e-07
## 646              chrIV.13850236_DSL21_103_0 3.240659e-07
## 978             chrIV.12804273_DSL21_95.1_0 8.526672e-57
## 1030                  chrIV.12816360_Ht31_1 1.542548e-53
## 1050            chrIV.12813908_DSL21_95.2_0 5.970614e-73
## 1051            chrIV.12802989_DSL21_95.4_0 1.008710e-63
## 1146    chrIV.12811933_Fg51_EDA.SNP120.233_1 7.039968e-81
## 1149            chrIV.12871886_DSL21_96.2_0 1.373142e-16
## 1406        chrIV.12815024_Fg6_EDA.PAM.10_1 1.396356e-71
## 1507                  chrIV.12810099_Hp43_1 1.076543e-56
## 1554                  chrIV.13850026_Hp50_1 2.892420e-07
## 1671 chrI.21689292_Fg2_ATP1A2.LG1.21Mb6914_1 1.469995e-05
```

Note: almost all these signficant markers are on Chromosome IV between ~12-14 Mb. This region contains a gene called *EDA* which, in humans, is associated with ectodermal abnormalties (ectodermal dysplasia). Even with slight deviations from the assumptions we get quite robust results. Several independent regions have shown this locus to be associated (and even causative) in controlling the number of lateral plates in threespine sticklebacks (eg Colosimo et al. 2015, *Science*).