Transition to R Classes 4 and 5: Basic Statistics – Regression, ANOVA, t-test
Tools for basic statistics in the base R package.

Goals:

Sample data sets:

The examples here draw on five data frames (rd, three, two, fb, nes), available from the course web page by running the following code in R.

```
#run this block of code to get all five data frames
rd<-
read.table("http://people.ucsc.edu/~ggilbert/Rclass_docs/RegressionDataset.csv",sep=",",heade
r=TRUE)

three<-
read.table("http://people.ucsc.edu/~ggilbert/Rclass_docs/ThreeTreatmentDataset.csv",sep=",",h
eader=TRUE)

two<-three[-which(three$treatment=="potassium"),]

fb<-
read.table("http://people.ucsc.edu/~ggilbert/Rclass_docs/factorialdata.csv",sep=",",header=TR
UE)

nes<-
read.table("http://people.ucsc.edu/~ggilbert/Rclass_docs/NestedAOVdata.csv",sep=",",header=TR
UE)
#end of code to get data frames
```

(1) Model Objects and Extractor Functions: When you run an analytical function in R, you usually get back a very sparse result, not enough for what you need. That is because the output of analytical functions like linear regression (lm) and analysis of variance (aov), are *model objects,* usually of type List and most of the model object are hidden from view. These model objects often have a large number of important embedded components that you can access using a variety of *extractor functions* (e.g., summary(), anova()) that package the information into useful structures and present the results.

Always assign the output of a model you run to an object and then extract it; don't just run the model.
Do this: `lmout<-lm(rd$temp~rd$precip)` *Not this:* `lm(rd$temp~red$precip)`

To see a "table of contents" of the model object, use the str() function.
```
lmout<-lm(rd$temp~rd$precip)
str(lmout) # this shows that lmout is a List with 12 elements
```

•You can access some components of the model object by name (e.g., `coefficients(lmout)`)
•You can use the $ to access named components (e.g., `lmout$coefficients`)
•You can call elements by the position in the list; e.g., since fitted.values are the 5th $ element in the model object list, so you can call
`lmout[[5]]  #the [[ ]] is used as position indicator in lists`
Some elements of the list have sub-elements; e.g., "`lmout$qr`" (element 7) has five $ sub-elements, the third of which is "`pivot`". There are several ways to see the values. Each of the following gives the same result:
`lmout$qr[3]` `lmout[[7]][3]` `lmout[[7]]$pivot` `lmout$qr$pivot`

Doing statistics in R is a matter of knowing which analytical functions are appropriate, what the structure of the resulting model object is, and which extractor functions and names are useful for getting what you want.

Generic extractor functions
There are a number of generic extractor functions that package the model output in useful ways.

Generic extractor	What it does
summary()	shows parameter estimates, and statistical values such as F, R^2, P, df
anova()	gives the ANOVA table with SS, MS, F, P, df
plot()	produces 4 diagnostic plots: Residual vs. Fitted; Normal QQ; Scale Location; Residual vs. leverage. Hit return in console to produce each in turn; scroll through with command-arrows. Or use this to see all at once: `par(mfrow=c(2,2));plot(myModel); par(mfrow=c(1,1))`
coef()	shows the estimated parameters from the fit model
fitted()	shows the fitted valued as predicted by the model for the independent variables included in the model
resid()	shows the residuals; measured minus predicted values of the dependent variable
predict()	produces a smooth function based on the fitted model to plot

`methods(class=`*function*`)` shows all the extractor functions available for a function (usually)

1B. A note on attach() and detach()

Attach(): a useful and *dangerous* friend to avoid typing the data frame name repeatedly
If you are only going to be working with one data frame for a while, and you don't want to have to type the dataframe$column1 each time, and instead just call column1, you can use the function attach.

Compare:

```
rdout<-lm(rd$temp~rd$precip)  #specify the data frame for each variable
plot(rd$temp~rd$precip)
```

with:

```
attach(rd) #attach the data frame
rdout<-lm(temp~precip) #call directly to the variables
plot(temp~precip)
detach(rd) #detach the data frame to work on a different data frame
```

Be careful with attach()! If you have a variables called "temp" and "precip" in data frame "rd" (rd$temp) and attach the data frame "rd", you then just call lm(temp~precip). BUT, if you happen to have another object in your workspace called simply "temp", things can get ugly very quickly. Use attach with caution, and be sure to detach as soon as appropriate.

1C. How to write formulas for models in R

Models in R take the form: *response variable ~ explanatory variables*

R formulas have several **special symbols**

*	interaction, e.g., A*B is A, B, and the interactions between A and B
^	interaction, e.g., (A+B)^2 is A, B, and the interactions between A and B
:	interaction, e.g., A:B is the interaction between A and B (compare to *)
/	nested, e.g., A/B is A + B nested in A, note that left to right is largest to smallest plot
I()	The "as is" function. Because *, /, and ^ are both operators (multiply, divide, to the power) AND they mean something different in formulas (interaction, nested, interaction), the I() allows what is inside the () to be treated as an operator. y~x + I(x^2) + I(1/z) says to fit $y = x + x^2 + 1/z$
Error()	Allows specification of error terms to use in models when there are multiple error terms, such as in split-plot designs
-	remove this factor from the model

If: y is a continuous dependent variable
x and z are continuous independent variables
A, B, and C are categorical factors

Model formulation	What it does
y ~ 1	#the null model of intercept only
y ~ x	# y is a function of x
y ~ x+z	#multiple regression with two independent variables
y ~ x*z	#multiple regression with interaction as y ~ x+z+x:z
y ~ (x+z)^2	# multiple regression with interaction as y ~ x+z+x:z
y ~ x + I(x^2) + z	# fits the model $y = x + x^2 + z$
y ~ poly(x,2) + z	# fits the model $y = x + x^2 + z$
y ~ x-1	#y as a function of x with no intercept (force through zero)
log(y) ~ I(1/x) + sqrt(z)	#fits transformed model $\ln(y) = 1/x + \sqrt{z}$
y ~ A	#one-way ANOVA
y ~ A+B	#two-way ANOVA
y ~ A*B	#2-way factorial ANOVA
y ~ A+B+A:B	#explicit form of 2-way factorial ANOVA
y ~ A*B*C-A:B:C	#3-way factorial ANOVA, but do not fit the 3-way interaction term
y ~ x+A	# analysis of covariance, with one slope, two intercepts (covariate first)
y ~ x*A	# analysis of covariance, with two slopes and two intercepts
y ~ A/B/C	#Factor C nested in B nested in A, left to right is largest to smallest
y ~ A + B %in% A	#A plus B nested in A, the equivalent of y ~ A/B
y ~ A*B*C+Error(A/B/C)	#Split-plot factorial with different error variances for each of 3 plot sizes

The update function. update() allows you to test reduced variations of a full model without re-writing all the terms each time. The term "~." (tilde period) means "the model as it is.

```
FullModel <- lm(y~A*B)  # fit the full model of y = A + B + A:B, and save as FullModel
NoIntModel<- update(FullModel,~. -A:B) #based on FullModel, fit the reduced model y = A + B.
```

(2) Linear regression using lm()

2A. Simple linear regression

Data frame rd includes data on mean temperature, mean precipitation, and the number of species (temp, precip, num_spp) in 30 plots.
Use this code to get it from the course website

```
rd<-
read.table("http://people.ucsc.edu/~ggilbert/Rclass_docs/RegressionDataset.csv",sep
=",",header=TRUE)

head(rd,4)
  temp   precip num_spp
1   15  7.55427       5
2    8 18.25095       3
3    6 27.53882       1
4   16 48.95995      11
```

Here are three ways to do the same thing: fit a simple linear regression of num_ssp on precip; (that is, *num_spp = B0 + B1(precip)*) and save the model object to slrout

```
#1. specify the data frame for each variable
slrout<-lm(rd$num_spp ~ rd$precip)

#2. specify the data frame once, then just call variables with simple names
slrout<-lm(data=rd, num_spp ~ precip)

#3. attach the data frame, then call the variables by simple names
attach(rd)
slrout<-lm(num_spp ~ precip)
detach(rd)  #do not forget to detach when you are done!
```

Remember, you have now created a model object called "slrout" with lots of component elements. You have to use extractor functions to see and use the different elements.

```
str(slrout) #shows the table of contents of the model object slrout
```

In abbreviated form, the main components are:

```
$ coefficients : Named num [1:2] 4.8781 0.0964
$ residuals    : Named num [1:30] -0.607 -3.638 -6.534 1.401 2.496 ...
$ effects      : Named num [1:30] -109 46.09 -5.81 2 3.04 ...
$ rank         : int 2
$ fitted.values: Named num [1:30] 5.61 6.64 7.53 9.6 10.5 ...
$ assign       : int [1:2] 0 1
$ qr           :List of 5 (qr, qraux, pivot, tol, rank)
$ df.residual  : int 28
$ xlevels      : list()
$ call         : language lm(formula = num_spp ~ precip)
$ terms        :Classes 'terms', 'formula' length 3 num_spp ~ precip
$ model        :'data.frame': 30 obs. of  2 variables:
  ..$ num_spp: int [1:30] 5 3 1 11 13 13 14 15 6 19 ...
  ..$ precip : num [1:30] 7.55 18.25 27.54 48.96 58.35 ...
```

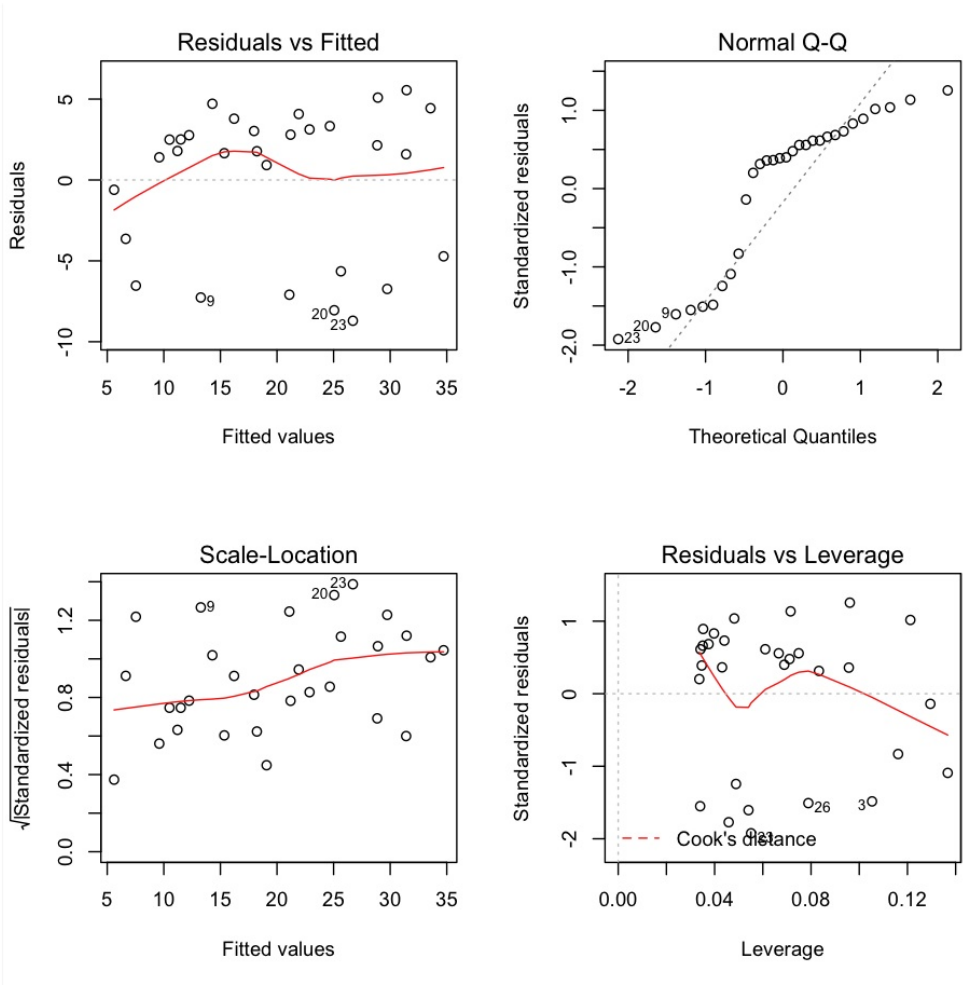
The following six lines will show you the most of what you need for simple linear regression.

```
slrout  #shows the model
summary(slrout) #this shows parameter estimates and errors, plus significance
anova(slrout) #gives the ANOVA table
par(mfrow=c(2,2)); plot(slrout); par(mfrow=c(1,1)) #4 diagnostic plots on 1 page
plot(rd$num_spp~rd$precip) #plot of the original data
abline(slrout)  #takes the fitted model, and draws that line through the data
```

HERE IS THE OUTPUT

```
> slrout  #shows the model

Call:
lm(formula = num_spp ~ precip)

Coefficients:
(Intercept)       precip
    4.87809      0.09643

> summary(slrout) #this shows parameter estimates and errors, plus significance

Call:
lm(formula = num_spp ~ precip)

Residuals:
   Min     1Q Median     3Q    Max
-8.703 -4.449  1.785  3.100  5.553

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 4.878094   1.738312   2.806  0.00902 **
precip      0.096429   0.009735   9.905 1.19e-10 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 4.653 on 28 degrees of freedom
Multiple R-squared: 0.778,    Adjusted R-squared:  0.77
F-statistic: 98.11 on 1 and 28 DF,  p-value: 1.187e-10

> anova(slrout) #gives the ANOVA table
Analysis of Variance Table

Response: num_spp
          Df  Sum Sq Mean Sq F value    Pr(>F)
precip     1 2124.42 2124.42  98.113 1.187e-10 ***
Residuals 28  606.28   21.65
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> par(mfrow=c(2,2)); plot(slrout); par(mfrow=c(1,1)) #diagnostic plots
> #note that the par statements just put the four plots on a sigle page
```

See next page for the graphs that appear in the quartz window (command-3)
Use command-left arrow to scroll back through graphs
Use command-right arrow to scroll forward through graphs



Resid vs. Fitted: points should not follow a pattern, and red lowess line should be relatively flat; if not might be missing an influential term in the model.

Normal Q-Q: Quantiles of standardized resides against those if data were normal; Should follow a straight line.

Scale-Location: If lowess line is flat, indicates homoscadasticity.

Resid vs. Leverage: Highlights points with strong influence on the fit of the model.

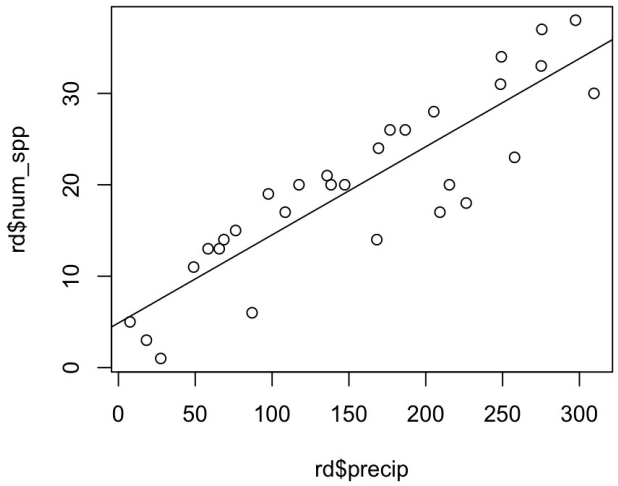


Figure 1. Number of species increases with greater mean precipitation.

2B. Accessing individual named components of lm model objects

`str(slrout)` #see the TOC of the 12 components embedded in the lm model object
In abbreviated form, the main components are:

```
$ coefficients : Named num [1:2] 4.8781 0.0964
$ residuals    : Named num [1:30] -0.607 -3.638 -6.534 1.401 2.496 ...
$ effects      : Named num [1:30] -109 46.09 -5.81 2 3.04 ...
$ rank         : int 2
$ fitted.values: Named num [1:30] 5.61 6.64 7.53 9.6 10.5 ...
$ assign       : int [1:2] 0 1
$ qr           :List of 5 (qr, qraux, pivot, tol, rank)
$ df.residual  : int 28
$ xlevels      : list()
$ call         : language lm(formula = num_spp ~ precip)
$ terms        :Classes 'terms', 'formula' length 3 num_spp ~ precip
$ model        :'data.frame': 30 obs. of  2 variables:
  ..$ num_spp: int [1:30] 5 3 1 11 13 13 14 15 6 19 ...
  ..$ precip : num [1:30] 7.55 18.25 27.54 48.96 58.35 ...
```

```
methods(class=lm) #shows the available extractor functions
 [1] add1.lm*           alias.lm*          anova.lm           case.names.lm*
 [5] confint.lm*        cooks.distance.lm* deviance.lm*       dfbeta.lm*
 [9] dfbetas.lm*        drop1.lm*          dummy.coef.lm*     effects.lm*
[13] extractAIC.lm*     family.lm*         formula.lm*        hatvalues.lm
[17] influence.lm*      kappa.lm           labels.lm*         logLik.lm*
[21] model.frame.lm     model.matrix.lm    plot.lm            predict.lm
[25] print.lm           proj.lm*           residuals.lm       rstandard.lm
[29] rstudent.lm        simulate.lm*       summary.lm         variable.names.lm*
[33] vcov.lm*
   Non-visible functions are asterisked   #THAT MEANS THEY DON'T RETURN ANYTHING
```

You can extract individual elements by calling these methods, or by addressing the elements of the model object list, depending on what you need. See page 2.
Try these, as examples:

```
formula(slrout)
extractAIC(slrout)
fitted(slrout)
coefficients(slrout)
slrout$coefficients
slrout[[1]]
slrout$model
slrout$call
slrout$df
```

You can extract data, statistics, and descriptors from the model to use in other analyses, to adorn graphics, or to create tables of values. For example, try this:

```
plot(rd$num_spp~rd$precip)
abline(slrout)
text(150,5,paste("num_spp=", round(slrout$coefficients["(Intercept)"],3), "+",
round(slrout$coefficients["precip"],3),"precip"),pos=4)
text(150,3,expression(R[adj]^2~"="),pos=4)
text(150,3,paste("          ",round(summary(slrout)$r.squared,3)),pos=4)
```

2C. Using predict() to get fitted lines and confidence intervals

The model object produced by lm() contains all the information to generate fitted values and confidence intervals for a line. The function predict() gives you those values *for each of the values of the independent variable used in creating the original model.* `predict()` takes the general form:

```
predict(modelobject, interval = c("none", "confidence", "prediction"), level = 0.95)
```

`Interval` can take one of three values:
"none" or blank: the predicted line itself
"confidence" or "c": CI that reflect uncertainty around the line itself; if level=0.95, traditional 95%CI
"prediction" or "p": PI reflect uncertainty about future observations

Predict takes a model fit from one set of data, and applies it across a desired ranges of x values in a separate data frame, but with the same variable name as used in the original model. The output includes three columns: the model fit, the lower CI, and the upper CI.

By default, predict uses the values for the dependent variable used to fit the original model to generate the fitted data or confidence intervals. If you want to predict over a different range of dependent variables, you can specify newdata. This takes the general form:

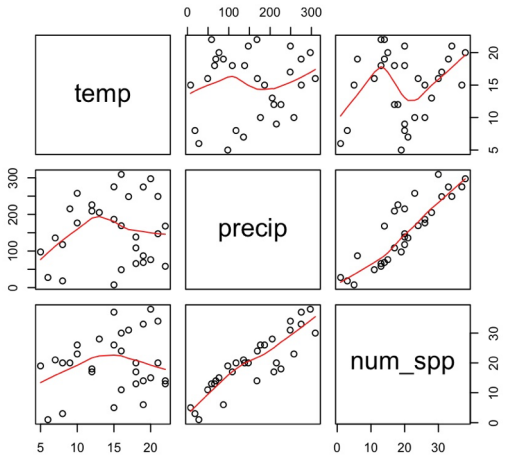
```
predict(modelobject, newdata=data.frame(originaldepvarname=yournewvalues))
```

```
#start with the linear regression of number of species on precipitation, as in 1A.
slrout<-lm(num_spp~precip, data=rd) #produces an lm model object called slrout
predfit<-predict(slrout)  #generates the fitted values for each of the original x
values from precip
#look at output where open are original data and closed are predicted
plot(num_spp~precip, data=rd); points(predfit~rd$precip,pch=19)

#if you want to fit data over a particular range
predfit2<-predict(slrout,newdata=data.frame(precip=seq(50,150,10)))
    #uses predict to generate fitted values over the range of 50 to 150 by step 10
#look at output where open are original data and closed are predicted
plot(num_spp~precip, data=rd); points(predfit2~seq(50,150,10),pch=19)
or showing predicted values as a line
plot(num_spp~precip, data=rd); points(predfit2~seq(50,150,10),type="l")

#you can also use predict to give confidence intervals
predCI95<-predict(slrout,interval="confidence", level=0.95) #95% Confidence Int.
predPI95<-predict(slrout,interval="p", level=0.95) #95% Prediction Int.

#Confidence interval is where mean of future observations are most like to occur
#Prediction interval is where future observations are most like to occur.

#Put the fitted line and both kinds of confidence intervals on one graph
plot(rd$precip,rd$num_spp)
lines(predfit2~ seq(50,150,10),lwd=5, lty=2) #fitted line over particular range
matlines(rd$precip, predCI95, lty=2,col="black")  #draws confidence bands
matlines(rd$precip, predPI95, lty=3,col="blue") #draws prediction bands
matlines(rd$precip, predfit, lty=1, col="red", lwd=1) #draws regression line
```

2D. Multiple Linear Regression and Stepwise Model Selection

Use data frame "rd" from 2A, check if precip and temp are both predictive of number of species. First, take a look at pairwise correlations among the three variables.

```
pairs(rd,panel=panel.smooth) #graphs
cor(rd) #correlation matrix
```




```
             temp    precip   num_spp
temp    1.0000000 0.1177029 0.1902117
precip  0.1177029 1.0000000 0.8820303
num_spp 0.1902117 0.8820303 1.0000000
```

Since there is no strong correlation between temp and precip, you could go ahead and include both on the right side of the lm model statement

```
mlrout<- lm(num_spp~precip+temp,data=rd)
summary(mlrout)
```

```
lm(formula = num_spp ~ precip + temp, data = rd)

Residuals:
   Min     1Q Median     3Q    Max
-8.264 -4.107  1.187  3.563  6.298

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 2.567401   2.937668   0.874    0.390
precip      0.095302   0.009812   9.713 2.64e-10      # precip is important
temp        0.166867   0.170939   0.976    0.338      # temp is not important

Residual standard error: 4.657 on 27 degrees of freedom
Multiple R-squared: 0.7855,   Adjusted R-squared: 0.7697
F-statistic: 49.45 on 2 and 27 DF,  p-value: 9.397e-10
```

You can use **AIC (Akaike Information Criterion**) to compare different models. First, by hand:

```
AIC(lm(num_spp~precip+temp+precip*temp,data=rd)) #full model with interaction
[1] 183.6184
AIC(lm(num_spp~precip+temp,data=rd)) #no interaction term
[1] 182.2800
AIC(update(mlrout,~. -temp)) #precipitation only, using the update function
[1] 181.3205
AIC(lm(num_spp~temp,data=rd)) #temperature only
[1] 225.3643
```

Rule of thumb: **Δ**AIC (model$_i$ – best model) < 2 suggests substantial support for the reduced model; **Δ**AIC between 3 and 7 suggests much less support, **Δ**AIC suggests little support for the model. Removing the interaction term or temp and the interaction term has a small, negative **Δ**AIC (181.3-183.6) suggesting that the reduced model (num_spp~precip) has a lot of support. Removing precip instead (leaving num_spp~temp) has a huge **Δ**AIC =225.4-183.6, suggesting there is no support for that model. Together, this suggests that *temp* contributes little, and the best model is numspp~precip.

R has an automated **stepwise selection function** `step`, that uses AIC scores to do stepwise selection of model terms, beginning with the full model. Automatically keeps the best model, given its cutoffs.

```
sd<-step(lm(num_spp~precip*temp,data=rd),direction="backward", trace=1)
summary(sd)
```

```
> sd<-step(lm(num_spp~precip*temp,data=rd),direction="backward", trace=1)

Start:  AIC=96.48                           #AIC for the Full model,
num_spp ~ precip * temp

              Df Sum of Sq    RSS    AIC
- precip:temp  1    12.773 585.61 95.144   #removes the interaction term
<none>                     572.84 96.482

Step:  AIC=95.14
num_spp ~ precip + temp

         Df Sum of Sq     RSS     AIC
- temp    1     20.67  606.28  94.184      #removes the temperature term
<none>                585.61  95.144      #AIC with precip + temp in model
- precip  1   2046.29 2631.90 138.228      #removes the precipitation term

Step:  AIC=94.18
num_spp ~ precip

         Df Sum of Sq     RSS     AIC
<none>                606.28  94.184      #AIC with just precipitation term
- precip  1    2124.4 2730.70 137.333      #AIC with intercept only

> summary(sd)                               #gives summary of best model

Call:
lm(formula = num_spp ~ precip, data = rd)  #structure of the best model

Residuals:
   Min     1Q Median     3Q    Max
-8.703 -4.449  1.785  3.100  5.553

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 4.878094   1.738312   2.806  0.00902
precip      0.096429   0.009735   9.905 1.19e-10

(Intercept) **
precip      ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 4.653 on 28 degrees of freedom
Multiple R-squared: 0.778,    Adjusted R-squared:  0.77
F-statistic: 98.11 on 1 and 28 DF,  p-value: 1.187e-10
```

Notes on optional parameters:
`Direction`: can take the values "backward", "forward", "both"
`trace`: if positive, gives information on each step. Larger values give more info.
`k`: allows you to change the number of degrees of freedom for the penalty

(3) Means comparisons: ANOVA & t-test

Get data frames three and two

```
three<-
read.table("http://people.ucsc.edu/~ggilbert/Rclass_docs/ThreeTreatmentDataset.csv"
,sep=",",header=TRUE)
two<-three[-which(three$treatment=="potassium"),]
```

3A. Independent, 2-sample t-test, test of equal variance, and wilcoxon rank test

First, the simplest ANOVA, an independent-sample t-test on data frame "two"
Two treatments (*control* and *nitrogen*), with 10 reps each; dependent variable *plant_mass*

#independent-sample t-test with unequal variances

```
t.test(plant_mass~treatment, data=two)
      Welch Two Sample t-test

data:  plant_mass by treatment
t = -16.3829, df = 15.237, p-value = 4.359e-11
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -2.446290 -1.883710
sample estimates:
 mean in group control mean in group nitrogen
                 2.479                  4.644
```

#Note - t.test() default is to assume unequal variances.
#to assume equal variances and pool them,

```
t.test(plant_mass~treatment, data=two, var.equal=T)
```

#Test whether the variances are different or not

```
var.test(plant_mass~treatment, data=two)
      F test to compare two variances

data:  plant_mass by treatment
F = 2.4831, num df = 9, denom df = 9, p-value = 0.1916
alternative hypothesis: true ratio of variances is not equal to 1
95 percent confidence interval:
 0.6167757 9.9970885
sample estimates:
ratio of variances
          2.483135
# p=0.19 so we cannot reject that the variances are equal
```

#non-parametric rank-test comparison of two independent samples

```
wilcox.test(plant_mass~treatment, data=two)
      Wilcoxon rank sum test with continuity correction

data:  plant_mass by treatment
W = 0, p-value = 0.0001806
alternative hypothesis: true location shift is not equal to 0

Warning message:
In wilcox.test.default(x = c(2.2, 2.64, 2.64, 2, 2.9, 2.28, 2.82,  :
  cannot compute exact p-value with ties
```

3B. Paired-sample and one-sample tests

Rearrange the data in "two" so that they are "paired" data (pretend they were collected that way)

```
cnpair<-data.frame(cbind(two[1:10,2],two[11:20,2]))
names(cnpair)<-c("control","nitrogen")
cnpair
```

```
   control nitrogen
1     2.20     4.68
2     2.64     4.84
3     2.64     4.20
4     2.00     4.77
5     2.90     4.38
6     2.28     4.79
7     2.82     4.77
8     2.36     4.83
9     2.93     4.43
10    2.02     4.75
```

```
t.test(cnpair$control, cnpair$nitrogen, paired=TRUE)  #paired t-test
```

```
      Paired t-test

data:  cnpair$control and cnpair$nitrogen
t = -13.4803, df = 9, p-value = 2.842e-07
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -2.528313 -1.801687
sample estimates:
mean of the differences
                 -2.165
```

```
wilcox.test(cnpair$control, cnpair$nitrogen, paired=TRUE)  #non-parametric test
```

```
      Wilcoxon signed rank test

data:  control and nitrogen
V = 0, p-value = 0.001953
alternative hypothesis: true location shift is not equal to 0
```

```
t.test(cnpair$control, mu=2.0)  #1-sample t-test, is control mean ≠ 2.0?
```

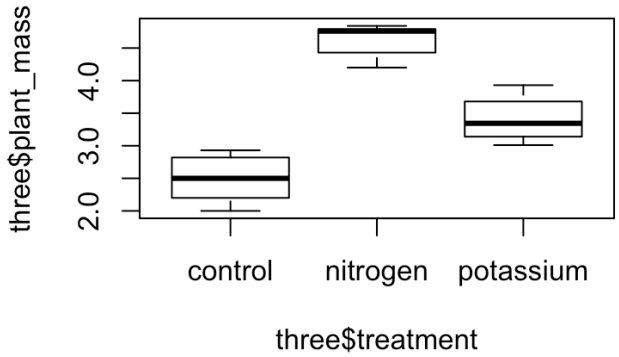
```
      One Sample t-test

data:  cnpair$control
t = 4.2929, df = 9, p-value = 0.002011
alternative hypothesis: true mean is not equal to 2
95 percent confidence interval:
 2.226591 2.731409
sample estimates:
mean of x
    2.479
```

3C. Simple one-way ANOVA (using dataframe "three")
There are a variety of ways to do Analysis of Variance in R.
Here we will look at the functions lm, aov, oneway.test, and the Kruskal-Wallace Test.

•lm and aov give identical results, but the output is slightly different.
•If you have multiple error terms, you must use aov.
•Some extractor functions, like Tukey HSD and model.tables are not compatible with lm.
•Overall, aov is probably more generally useful for ANOVAS.
•oneway.test is a variant that does not assume equal variances across groups.
•kruskal.test is a non-parametric comparison among groups.
•If you want to do mixed-models and specify fixed and random effects, use lmer in lme4 package.

First, take a quick look at data means and variance measures using box-and-whisker plots

```
plot(three$plant_mass~three$treatment)
```



How to read a box-and-whisker plot: The thick line is the median; The upper and lower part of the box are the 25% and 75% percentiles (1st and 3rd quantiles); The whiskers show either the maximum and minimum values OR 1.5X the interquartile range (~2 standard deviations), whichever is smaller. If the latter, outlier points are shown individually.

Using the "lm" approach to ANOVA

```
attach(three)
a3<-lm(plant_mass~treatment) #run an ANOVA using lm and put in a3
anova(a3); summary(a3) #show basic info from model object
pairwise.t.test(plant_mass,treatment) #LSD pairwise post-hoc
meansa3<-tapply(plant_mass,treatment, mean) #get the means for each treatment
sda3<-tapply(plant_mass,treatment, sd) #get the sd for each treatment
as.table(cbind(meansa3,sda3)) #put means and sd into pretty table
detach(three)
```

#NOTE: SEE THE NEXT PAGE FOR SOME HELP IN INTERPRETATION OF THE OUTPUT

```
Analysis of Variance Table

Response: plant_mass
          Df  Sum Sq Mean Sq F value    Pr(>F)
treatment  2 23.5891 11.7946  121.15 3.274e-14 ***
Residuals 27  2.6285  0.0974
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Call:
lm(formula = plant_mass ~ treatment)

Residuals:
    Min      1Q  Median      3Q     Max
-0.4790 -0.2630  0.0730  0.1797  0.5200

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)         2.47900    0.09867  25.125  < 2e-16 ***
treatmentnitrogen   2.16500    0.13954  15.516 5.66e-15 ***
treatmentpotassium  0.93100    0.13954   6.672 3.68e-07 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.312 on 27 degrees of freedom
Multiple R-squared: 0.8997,   Adjusted R-squared: 0.8923
F-statistic: 121.2 on 2 and 27 DF,  p-value: 3.274e-14

> pairwise.t.test(plant_mass,treatment) #LSD pairwise post-hoc

      Pairwise comparisons using t tests with pooled SD

data:  plant_mass and treatment

          control nitrogen
nitrogen  1.7e-14 -
potassium 3.7e-07 3.7e-09

P value adjustment method: holm
> meansa3<-tapply(plant_mass,treatment, mean) #get the means for each treatment
> sda3<-tapply(plant_mass,treatment, sd) #get the sd for each treatment
> as.table(cbind(meansa3,sda3)) #put means and sd into pretty table
            meansa3      sda3
control   2.4790000 0.3528440
nitrogen  4.6440000 0.2239147
potassium 3.4100000 0.3426693
```

Interpreting summary() and anova() estimates from lm() ANOVA models in R

In the data frame three used in the example above, we are looking at an experiment with one treatment factor that has three levels: control, nitrogen, and potassium. Above we created the model object called "a3" from lm(plant_mass~treatment).

In many statistical packages, we get a measure of the significance of the overall effect of treatment, including all the levels. This kind of output is extracted using anova() or summary.aov()

```
> anova(a3)
            Df  Sum Sq Mean Sq F value    Pr(>F)
treatment    2 23.5891 11.7946  121.15 3.274e-14 ***
Residuals   27  2.6285  0.0974
```

However, when we used lm() to create a ANOVA model object the summary(a3) output looks like this (in part):

```
Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
(Intercept)         2.47900    0.09867  25.125  < 2e-16 ***
treatmentnitrogen   2.16500    0.13954  15.516 5.66e-15 ***
treatmentpotassium  0.93100    0.13954   6.672 3.68e-07 ***
```

In a regression, the Coefficient estimates are easy to interpret – they are the coefficients for the regression equation, starting with the intercept, and then a coefficient for each term in the model (num_spp ~ temp + precip would give three coefficient estimates – one intercept and one each for temp and precip). For this ANOVA, we use the simple model lm(plant_mass~treatment) but get three coefficients. That is because R defaults to *treatment contrasts* in presenting output from lm() anova models. Recall that the underlying model fit by lm(plant_mass~treatment) is really:

$$plant\_mass = a + b*treatment_1 + c*treatment_2 + d* treatment_3$$

By convention, the mean for whatever treatment level comes first in your data frame (in this case, *control*) becomes the coefficient estimate for the Intercept. The other estimates are the differences between this mean and the other means of treatment levels.

So: to get the mean value of nitrogen, it would be 2.479+2.165 = 4.644.
The mean value of potassium would be 2.479+0.931 = 3.41

Recall from above that when we calculated group means using tapply, we got:

```
             means        sd
control   2.4790000 0.3528440
nitrogen  4.6440000 0.2239147
potassium 3.4100000 0.3426693
```

You can control which is the reference level using "relevel"

```
fb$treatment<-relevel(fb$treatment,ref="potassium")
summary(lm(fb$plant_mass~fb$cultivar*fb$treatment))
```

Using the "aov" approach to ANOVA

```
attach(three)
a4<-aov(plant_mass~treatment)
a4;anova(a4);summary.lm(a4);
TukeyHSD(a4) #does not work for lm
model.tables(a4,"means",se=T,n=T) #does not work for lm
meansa4<-tapply(plant_mass,treatment, mean)
sda4<-tapply(plant_mass,treatment, sd); as.table(cbind(meansa4,sda4))
detach(three)
```

We'll just look at a couple things here, rather than show the full output. When using aov() for anova, the summary() and anova() extractors function identically, but differently from what summary() give you for an object created using lm(). The extractor function summary.lm() gives the treatment-contrast approach discussed above.

anova() provides a test of the overall significance of treatment (all the levels included)

```
anova(a4)
Analysis of Variance Table

Response: plant_mass
          Df  Sum Sq Mean Sq F value    Pr(>F)
treatment  2 23.5891 11.7946  121.15 3.274e-14 ***
Residuals 27  2.6285  0.0974
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

summary.lm() provides information about the significance of the effect of each treatment level, as described in the previous section.

```
summary.lm(a4)
Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
(Intercept)         2.47900    0.09867  25.125  < 2e-16 ***
treatmentnitrogen   2.16500    0.13954  15.516 5.66e-15 ***
treatmentpotassium  0.93100    0.13954   6.672 3.68e-07 ***

Residual standard error: 0.312 on 27 degrees of freedom
Multiple R-squared: 0.8997,   Adjusted R-squared: 0.8923
F-statistic: 121.2 on 2 and 27 DF,  p-value: 3.274e-14
```

Note that several other useful extractor functions, like TukeyHSD() and model.tables() work for model objects created using aov() but not for lm().

```
TukeyHSD(a4)   #provides a conservative post-hoc comparison of treatment means
  Tukey multiple comparisons of means
$treatment
                     diff        lwr        upr   p adj
nitrogen-control    2.165  1.8190293  2.5109707 0.0e+00
potassium-control   0.931  0.5850293  1.2769707 1.1e-06
potassium-nitrogen -1.234 -1.5799707 -0.8880293 0.0e+00
```

Overall, doing ANOVAS using aov() is probably a bit more generally useful because there are more analytical options .

3D. Using the "oneway.test" approach to ANOVA

This is a one-way analysis of variance that does not assume equal variances

```
a5<-oneway.test(plant_mass~treatment, data=three)
> a5
      One-way analysis of means (not assuming equal variances)

data:  plant_mass and treatment
F = 140.9555, num df = 2.000, denom df = 17.123, p-value = 2.319e-11
```

#note – this is pretty much all that is available There is not much to extract.

```
str(a5)
List of 5
 $ statistic: Named num 141
  ..- attr(*, "names")= chr "F"
 $ parameter: Named num [1:2] 2 17.1
  ..- attr(*, "names")= chr [1:2] "num df" "denom df"
 $ p.value  : num 2.32e-11
 $ method   : chr "One-way analysis of means (not assuming equal variances)"
 $ data.name: chr "plant_mass and treatment"
 - attr(*, "class")= chr "htest"
```

Using the non-parametric "kruskal.test" approach to ANOVA

Here is the non-parametric Kruskal-Wallace test for differences among groups

```
a6<-kruskal.test(plant_mass~treatment, data=three)
a6
      Kruskal-Wallis rank sum test

data:  plant_mass by treatment
Kruskal-Wallis chi-squared = 25.8179, df = 2, p-value = 2.476e-06


#As for oneway.test, that is pretty much it.  Not much to extract.
str(a6)
List of 5
 $ statistic: Named num 25.8
  ..- attr(*, "names")= chr "Kruskal-Wallis chi-squared"
 $ parameter: Named num 2
  ..- attr(*, "names")= chr "df"
 $ p.value  : num 2.48e-06
 $ method   : chr "Kruskal-Wallis rank sum test"
 $ data.name: chr "plant_mass by treatment"
 - attr(*, "class")= chr "htest"
```

3E. Factorial ANOVAS

```
fb<-
read.table("http://people.ucsc.edu/~ggilbert/Rclass_docs/factorialdata.csv",sep=","
,header=TRUE)     # experiment with two cultivars and three treatments, 10 reps of each combination.
```

For this part, imagine it is a completely randomized design (i.e., ignore "block")

```
attach(fb)
a6<-aov(plant_mass~cultivar*treatment) #set up as a factorial using *
a6; anova(a6); summary.lm(a6)
model.tables(a6,"means",se=TRUE)
TukeyHSD(a6)
interaction.plot(cultivar,treatment,plant_mass)
detach(fb)
```

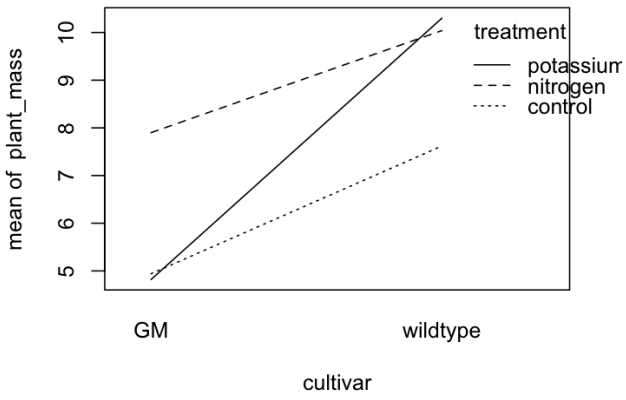
In this case, anova(a6) shows that overall, there is only a marginally significant effect of the interaction term, but that both cultivar and treatment have main significant main effects.

```
Response: plant_mass
                   Df  Sum Sq Mean Sq F value   Pr(>F)
cultivar            1 129.067 129.067 75.1196 8.39e-12 ***
treatment           2  72.817  36.409 21.1906 1.61e-07 ***
cultivar:treatment  2   8.945   4.473  2.6032   0.0833 .
Residuals          54  92.780   1.718
```

summary.lm(a6) breaks it down into treatment contrasts, and shows that the effect of treatment is due entirely to nitrogen.

```
                                  Estimate Std. Error t value Pr(>|t|)
(Intercept)                         4.9400     0.4145  11.918  < 2e-16 ***
cultivarwildtype                    2.6800     0.5862   4.572 2.86e-05 ***
treatmentnitrogen                   2.9600     0.5862   5.049 5.38e-06 ***
treatmentpotassium                  0.8800     0.5862   1.501    0.139
cultivarwildtype:treatmentnitrogen -0.5400     0.8290  -0.651    0.518
cultivarwildtype:treatmentpotassium 1.3000     0.8290   1.568    0.123
```

```
model.tables(a6) gives you a quick summary of means, standard errors, and n
Tables of means
Grand mean
7.686667
cultivar
      GM wildtype
   6.220    9.153

treatment
  control  nitrogen potassium
     6.28      8.97      7.81

 cultivar:treatment
          treatment
cultivar   control nitrogen potassium
  GM        4.94    7.90     5.82
  wildtype  7.62   10.04     9.80

Standard errors for differences of means
        cultivar treatment cultivar:treatment
          0.3384    0.4145             0.5862
replic.       30        20                 10
```



3F. Blocked or split-plot ANOVAS

```
fb<-
read.table("http://people.ucsc.edu/~ggilbert/Rclass_docs/factorialdata.csv",sep=","
,header=TRUE)
```

Experiment with two cultivars and three treatments, arranged in 10 complete blocks with a split-plot design. Each block is split in two, with one half sown to GM and half to wildtype. Each cultivar is then split in three, and receives nitrogen, potassium, or control.

Thus, each block has all combinations of 2x3 within it.
The largest plot size (block) is split with cultivar treatments; each cultivar is then split with one of three fertilizer treatments.

The treatments (cultivar and treatment) are coded as a factorial, either as `cultivar*treatment` or `(cultivar+treatment)^2`. The blocked, split-plot design requires specifying the error terms explicitly, start from large to small, but not including the smallest unit.

Thus: `aov(plant_mass~(cultivar + treatment)^2 + Error(block/cultivar))`

Note that not all the usual extractor functions work when the Error terms are specified.

```
attach(fb)
a7<-aov(plant_mass~(cultivar + treatment)^2 + Error(block/cultivar))
a7; summary(a7) #note: anova() and summary.lm() don't work for this kind of model
interaction.plot(cultivar,treatment,plant_mass)
detach(fb)
```

3G. Nested ANOVA

```
nes<-
read.table("http://people.ucsc.edu/~ggilbert/Rclass_docs/NestedAOVdata.csv",sep=","
,header=TRUE)
```

Comparison of native vs. exotic species of clover, with three species nested within each origin; 12 individuals of each species; dependent variable is percent survival.

General format for nesting is y~A/B/C, going from largest to smallest left to right.
For nes, where species is nested within origin:

```
nes<-nes[order(nes$origin,nes$species),]  #sort data frame by origin and species
a8<-lm(survival~origin/species, data=nes)
a8; anova(a8); summary(a8)
```

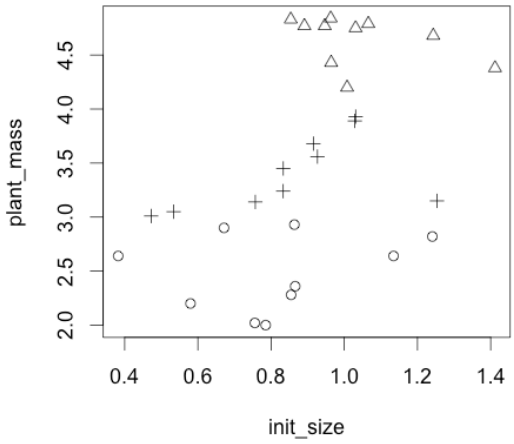
3H. ANCOVA (Analysis of Covariance)

```
three<-
read.table("http://people.ucsc.edu/~ggilbert/Rclass_docs/ThreeTreatmentDataset.csv"
,sep=",",header=TRUE)
```

Three treatments (control, nitrogen, potassium), with plants each. Measured the initial size (the covariate) and the plant_mass at the end of the experiment. Treatment is a categorical factor, and init_size is a continuous variable.

First take a look at how the dependent variable plant_mass is associated with the covariate init_size. Use pch to give each treatment a different symbol.

```
plot(plant_mass~init_size,
pch=as.numeric(treatment),data=three)
```



It looks like plants that started off larger ended up larger in the end. It is important to test whether any effect of treatment might be a simple product of initial size, or whether there is an interaction between treatment and initial size. Analysis of covariance to test whether there is an effect of treatment on biomass, after removing the effect of initial size. We will use AIC to test whether the interaction and covariate are important in the model.

To start with the full model, use the * to include interactions, and then fit each of the other reduced models. Note that the order of the terms in the model matters – the covariate comes first, and then the factors. Then use AIC to select the best model.

```
Lout1<-lm(plant_mass~init_size*treatment, data=three)
Lout2<-lm(plant_mass~init_size + treatment, data=three)
Lout3<-lm(plant_mass~treatment, data=three)
Lout4<-lm(plant_mass~init_size, data=three)
AIC(Lout1); AIC(Lout2); AIC(Lout3); AIC(Lout4)
```

The resulting AIC values are 20.39, 20.35, 20.09, and 80.12 respectively. Models 1,2, and 3 are not different from each other; the simplest model, then is Lout3, which only includes treatment. You can get to the same place using step(Lout1). You can also compare models using ANOVA.

```
anova(Lout1,Lout2,Lout3,Lout4)
Analysis of Variance Table

Model 1: plant_mass ~ init_size * treatment
Model 2: plant_mass ~ init_size + treatment
Model 3: plant_mass ~ treatment
Model 4: plant_mass ~ init_size
  Res.Df     RSS Df Sum of Sq        F    Pr(>F)
1     24  2.1740
2     26  2.4799 -2   -0.3058   1.6880    0.2061
3     27  2.6285 -1   -0.1487   1.6411    0.2124
4     28 20.7803 -1  -18.1517 200.3833 3.807e-13 ***
```

Use anova() and summary() to extract the most useful information from the model object.

(4) Testing for homogeneity of variance among groups

Testing for homogeneity of variance among groups for ANOVAS
There are two common tests for homogeneity of variance, Bartlett and Fliger-Killen

```
attach(three)
bartlett.test(plant_mass~treatment)
fligner.test(plant_mass~treatment)
detach(three)
```

```
> bartlett.test(plant_mass~treatment)

      Bartlett test of homogeneity of variances

data:  plant_mass by treatment
Bartlett's K-squared = 1.9741, df = 2, p-value = 0.3727

> fligner.test(plant_mass~treatment)

      Fligner-Killeen test of homogeneity of variances

data:  plant_mass by treatment
Fligner-Killeen:med chi-squared = 3.6421, df = 2, p-value = 0.1619
```

#Both tests indicate no significant difference in variance among groups

(5) Type I and III Sums of Squares

There is a raging debate over the appropriate uses of Type I, Type II, or Type III sums of squares. Type III SS have become quite common in Ecology, largely as a legacy of the way SAS handles things. By default, though, R uses Type I (sequential) sums of squares. This is an issue for studies with unbalanced designs – in balanced designs, Type I and III are the same. We are not going to discuss the relative merits in this class. The Car package in R is designed to handle different types of SS easily -- check it out. There is a lot of discussion on the web on different approaches. However, here is how you can do it in R base package.

```
#Create an unbalanced version of the fb (factorial) data frame from above
fbu<-fb[-c(6,16,18,33,34,59),]

# To get ANOVA table with Type I Sums of Squares
fbout<-lm(plant_mass~cultivar*treatment, data=fbu)
anova(fbout)

#to get Type III SS:
options(contrasts=c("contr.sum","contr.poly"))  # chooses contrast settings that
sum to zero
fbout2<-lm(plant_mass~cultivar*treatment, data=fbu)
drop1(fbout2, .~., test="F")
```

#Note: if instead you run these two versions using fb data frame, the ANOVA tables are identical

Operators

```
* interaction     : interaction     / nested    ^ interaction     I() as is
~.  the model as it is        Error() specify error terms
```

Linear regression models

```
Lout<-lm(DepVar~IndepVar, data=Mydata) #simple linear regression
Lout; anova(Lout); summary(Lout); plot(Lout)  #extract model object
predict(Lout, interval = "confidence, level = 0.95) #95%CI
```

ANOVA models

```
Aout<-aov(DepVar~Factor, data=Mydata)  #one-way ANOVA
Aout<-aov(DepVar~FactorA*FactorB, data=Mydata) #two-way factorial ANOVA
Aout<-aov(DepVar~FactorA + FactorB + FactorA:FactorB, data=Mydata) #2-way
Aout<-aov(DepVar~(FactorA+FactorB)^2, data=Mydata) #two-way factorial ANOVA
Aout<-aov(DepVar~Factor + Block, data=Mydata) #Randomized block ANOVA
Aout; anova(Aout); summary.lm(Aout);TukeyHSD(Aout) #extract model object
```

t-tests

```
t.test(DepVar~Factor, data=Mydata) #independent sample t-test
t.test(DepVar~Factor, data=Mydata, var.equal=T) #ind-sample t-test, pooled variance
t.test(DepVar, mu=2.0)  #one-sample t-test that mean is different from 2.0
t.test(DepVar1,DepVar2, data=Mydata, paired=TRUE) #paired sample t-test
wilcox.test(DepVar~Factor, data=Mydata) #wilcoxon rank-sum test
```

Testing assumptions

```
var.test(DepVar~Factor, data=Mydata) # test if two variances are equal
bartlett.test(DepVar~Factor,data=Mydata) #Bartlett test of homogeneity of variances
fligner.test(DepVar~Factor,data=Mydata) ##Fligner-Killeen test homog. of variance
cor(Mydata[col:col], method="pearson") #pairwise correlation coefficients (method =
    pearson, spearman, or kendall)
cor.test(Var1,Var2, method="spearman") #test of correlation between two variables
```

ANCOVA models

```
Lout<-lm(DepVar~Covar*Factor, data=Mydata) #Analysis of Covariance ANCOVA
```

Stepwise regression

```
step(lm(DepVar~IVar1+IVar2+IVar1*Ivar2, data=Mydata),direction="backward", trace=1)
AIC(modelobject)  #gives AIC values
```

Plotting

```
boxplot(DepVar~Factor, data=Mydata) #Boxplot of means across groups
coplot(DepVar~CoVar|Factor, data=Mydata) #visualize covariance across factors
plot(DepVar~IndepVar); abline(lm(DepVar~IndepVar, data=Mydata)); CI95<-
   predict(Lout,int="c",level=0.95); matlines(IndepVar, CI95, lty=2) #plot w 95%CI
pairs(Mydata[col:col]) #pair-wise scatterplots of variables in a data frame
```

Post-hoc tests

```
TukeyHSD(Aout) #post-hoc HSD comparison of means from model object
pairwise.t.test(DepVar,Factor) #post-hoc LSD comparison of means
```