Two-Way ANOVA

R Handout

Derek H. Ogle

```
> options(show.signif.stars=FALSE)
> library(NCStats)
> library(multcomp)     # for glht()
```

Bacteria Example

What is the optimal temperature (27,35,43C) and concentration (0.6,0.8,1.0,1.2,1.4% by weight) of the nutrient, tryptone, for culturing the *Staphylococcus aureus* bacterium. Each treatment was repeated twice. The number of bacteria (millions Colony Forming Units (CFU)/mL) was recorded in Bacteria.csv.

```
> setwd("C:/aaaWork/Web/GitHub/NCMTH207/modules/Anova-2Way")
> bact <- read.csv("Bacteria.csv")
> str(bact)
```

```
'data.frame':   30 obs. of  3 variables:
 $ temp : int  27 27 27 27 27 35 35 35 35 35 ...
 $ conc : num  0.6 0.8 1 1.2 1.4 0.6 0.8 1 1.2 1.4 ...
 $ cells: int  55 120 186 260 151 82 166 179 223 178 ...
```

```
> bact$temp <- factor(bact$temp)
> bact$conc <- factor(bact$conc)
```

```
> ylbl <- "Mean Number of Cells"
> conclbl <- "Concentration (%)"
> templbl <- "Temperature (C)"
```

Initial Summaries

```
> Summarize(cells~temp*conc,data=bact,digits=0)
```

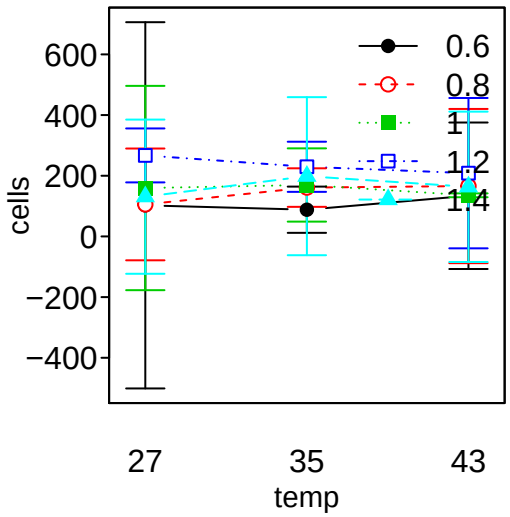
```
   temp conc n mean sd min  Q1 median  Q3 max
1    27  0.6 2  102 67  55  79    102 126 150
2    35  0.6 2   88  8  82  85     88  91  94
3    43  0.6 2  134 27 115 124    134 144 153
4    27  0.8 2  106 21  91  98    106 113 120
5    35  0.8 2  161  7 156 158    161 164 166
6    43  0.8 2  166 28 146 156    166 176 186
7    27    1 2  160 37 133 146    160 173 186
8    35    1 2  170 13 160 165    170 174 179
9    43    1 2  136  1 135 135    136 136 136
10   27  1.2 2  267 10 260 264    267 270 274
11   35  1.2 2  230  9 223 226    230 233 236
12   43  1.2 2  208 28 189 199    208 218 228
13   27  1.4 2  131 28 111 121    131 141 151
14   35  1.4 2  198 29 178 188    198 209 219
15   43  1.4 2  164 28 144 154    164 173 183
```

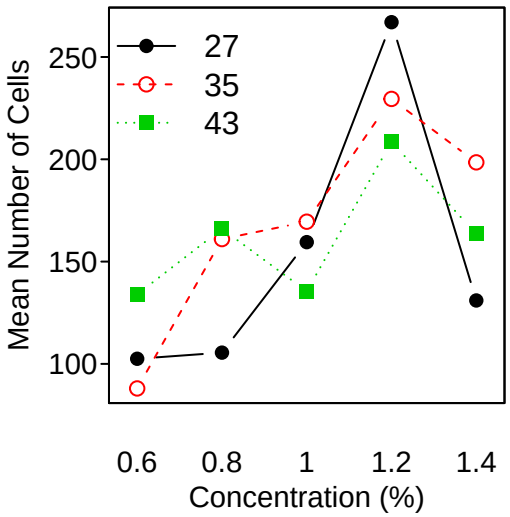
Model Fitting and Summary

```
> lm1 <- lm(cells~temp*conc,data=bact)
> anova(lm1)
```

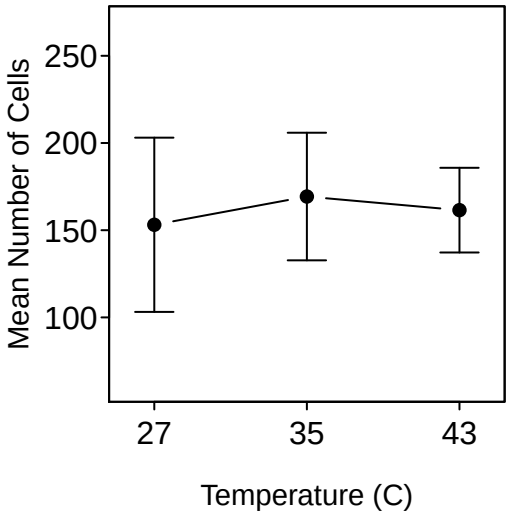
```
          Df Sum Sq Mean Sq F value    Pr(>F)
temp       2   1313   656.4  0.8557   0.44473
conc       4  51596 12899.1 16.8154 2.041e-05
temp:conc  8  14703  1837.8  2.3958   0.06886
Residuals 15  11507   767.1
```

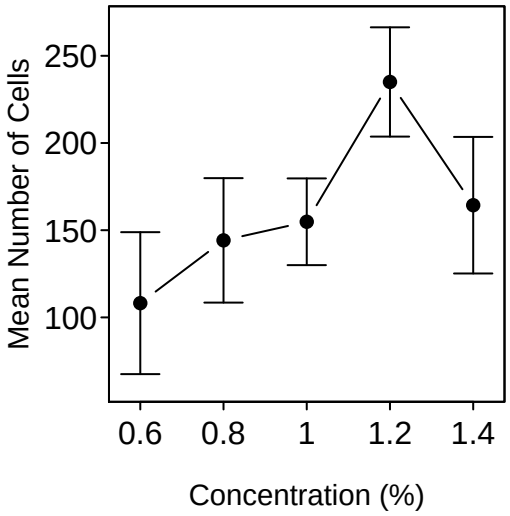
```
> fitPlot(lm1)  # left
> fitPlot(lm1,interval=FALSE,change.order=TRUE,xlab=conclbl,ylab=ylbl,legend="topleft")
```






```
> fitPlot(lm1,which="temp",ylim=c(60,270),xlab=templbl,ylab=ylbl)  # left
> fitPlot(lm1,which="conc",ylim=c(60,270),xlab=conclbl,ylab=ylbl)
```



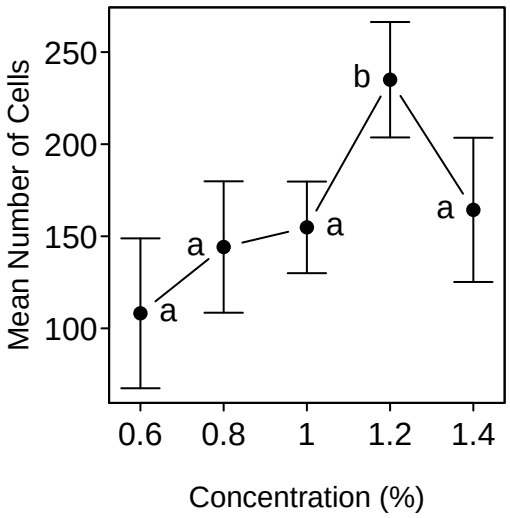


Multiple Comparisons

```
> bact.mc1 <- glht(lm1,mcp(conc="Tukey"))
> summary(bact.mc1)
```

```
               Estimate Std. Error    t value      p value
0.8 - 0.6 = 0      3.0   27.69657  0.1083167 0.9999648903
1 - 0.6 = 0       57.0   27.69657  2.0580166 0.2872759080
1.2 - 0.6 = 0    164.5   27.69657  5.9393636 0.0002462630
1.4 - 0.6 = 0     28.5   27.69657  1.0290083 0.8381774286
1 - 0.8 = 0       54.0   27.69657  1.9496999 0.3350483881
1.2 - 0.8 = 0    161.5   27.69657  5.8310469 0.0002615302
1.4 - 0.8 = 0     25.5   27.69657  0.9206916 0.8845090262
1.2 - 1 = 0      107.5   27.69657  3.8813470 0.0109916643
1.4 - 1 = 0      -28.5   27.69657 -1.0290083 0.8381765225
1.4 - 1.2 = 0   -136.0   27.69657 -4.9103553 0.0015469390
```

```
> fitPlot(lm1,which="conc",xlab=conclbl,ylab=ylbl)
> addSigLetters(lm1,which="conc",lets=c("a","a","a","b","a"),pos=c(4,2,4,2,2))
```



Soil Phosphorous Example

Soil phosphorous is important for the invasion of native vegatation by exotic weeds. Clements (1983) studied the soil phosphorous near Sydney (Australia) to determine how soil phosphorous varied with topographical location and soil type. Bushland sites were chosen in Brisbane Waters National Park, Ku-ring-gai Chase National Park, and Royal National Park as these areas were relatively unaffected by suburban development, were free from immediate roadside or track effects, and had not been burned for at least two years. Shale-derived and sandstone-derived soils in four topographic locations were examined with three 250 m2 quadrats in each of the eight combinations of soil type and topography. Cores of soil of 75 mm depth and 25 mm diameter, free from surface litter, were collected from each of five randomly selected points in each quadrat. The five soil samples were pooled and the total soil phosphorous (ppm) was determined for each pooled sample. Determine the effect of soil type and topography on total soil phosphorous level.

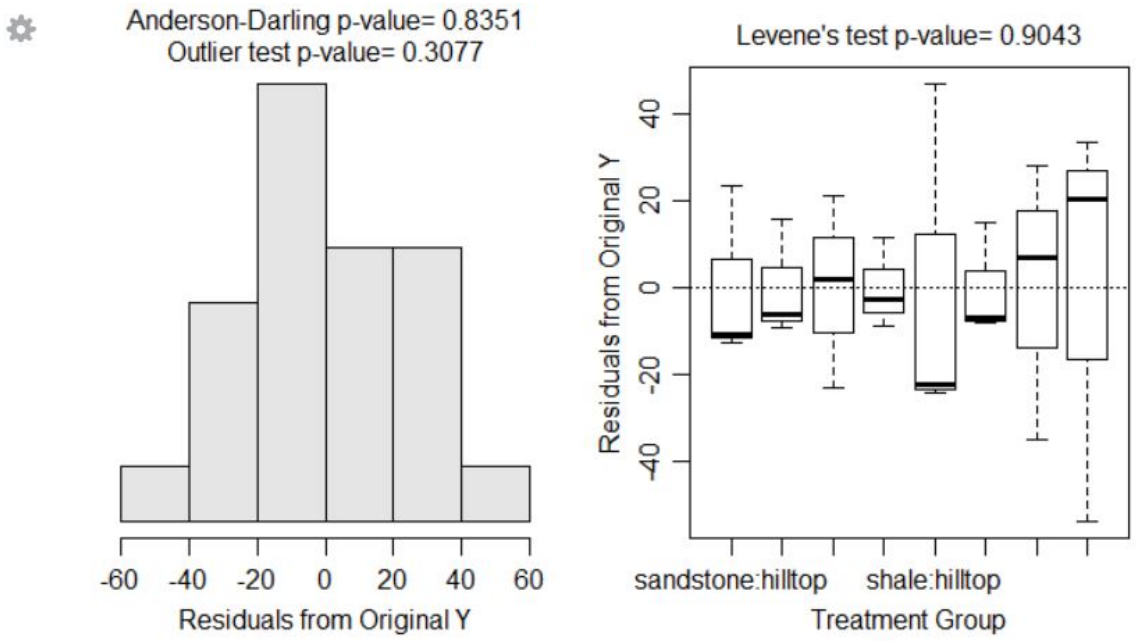
```
> sp <- read.csv("SoilPhosphorous.csv")
> str(sp)
```

```
'data.frame':   24 obs. of  3 variables:
 $ soil: Factor w/ 2 levels "sandstone","shale": 2 2 2 2 2 2 2 2 2 2 ...
 $ topo: Factor w/ 4 levels "hilltop","north",..: 4 4 4 2 2 2 3 3 3 1 ...
 $ phos: int  98 172 185 78 77 100 117 54 96 83 ...
```

Analysis

```
> lm1 <- lm(phos~soil*topo,data=sp)
```

```
> transChooser(lm1)
```




```
> anova(lm1)
```

```
          Df  Sum Sq Mean Sq F value    Pr(>F)
soil       1 17876.0 17876.0 22.9818 0.0001988
topo       3  9693.8  3231.3  4.1542 0.0235128
soil:topo  3 11390.8  3796.9  4.8814 0.0134826
Residuals 16 12445.3   777.8
```

```
> sp$comb <- sp$soil:sp$topo
> headtail(sp)
```

```
        soil    topo phos              comb
1      shale  valley   98      shale:valley
2      shale  valley  172      shale:valley
3      shale  valley  185      shale:valley
22 sandstone hilltop   55 sandstone:hilltop
23 sandstone hilltop   21 sandstone:hilltop
24 sandstone hilltop   19 sandstone:hilltop
```

```
> lm1a <- lm(phos~comb,data=sp)
> anova(lm1a)
```

```
          Df Sum Sq Mean Sq F value    Pr(>F)
comb       7  38961  5565.8  7.1555 0.0005729
Residuals 16  12445   777.8
```

```
> spint.mc <- glht(lm1a, mcp(comb="Tukey"))
> summary(spint.mc)
```

```
                                          Estimate Std. Error     t value     p value
sandstone:north - sandstone:hilltop = 0    1.666667   22.77181  0.07318989 0.999999999
sandstone:south - sandstone:hilltop = 0   19.333333   22.77181  0.84900274 0.986833482
sandstone:valley - sandstone:hilltop = 0  -4.000000   22.77181 -0.17565574 0.999999583
shale:hilltop - sandstone:hilltop = 0      4.666667   22.77181  0.20493170 0.999998782
shale:north - sandstone:hilltop = 0       53.333333   22.77181  2.34207653 0.329916887
shale:south - sandstone:hilltop = 0       57.333333   22.77181  2.51773227 0.255481523
shale:valley - sandstone:hilltop = 0     120.000000   22.77181  5.26967219 0.001471795
sandstone:south - sandstone:north = 0     17.666667   22.77181  0.77581285 0.992184512
sandstone:valley - sandstone:north = 0    -5.666667   22.77181 -0.24884563 0.999995346
shale:hilltop - sandstone:north = 0        3.000000   22.77181  0.13174180 0.999999943
shale:north - sandstone:north = 0         51.666667   22.77181  2.26888664 0.365316750
shale:south - sandstone:north = 0         55.666667   22.77181  2.44454238 0.284894037
shale:valley - sandstone:north = 0       118.333333   22.77181  5.19648230 0.001733065
sandstone:valley - sandstone:south = 0   -23.333333   22.77181 -1.02465848 0.963511327
shale:hilltop - sandstone:south = 0      -14.666667   22.77181 -0.64407105 0.997460536
shale:north - sandstone:south = 0         34.000000   22.77181  1.49307379 0.800343137
shale:south - sandstone:south = 0         38.000000   22.77181  1.66872953 0.705175648
shale:valley - sandstone:south = 0       100.666667   22.77181  4.42066945 0.007753747
shale:hilltop - sandstone:valley = 0       8.666667   22.77181  0.38058744 0.999916810
shale:north - sandstone:valley = 0        57.333333   22.77181  2.51773227 0.255502242
shale:south - sandstone:valley = 0        61.333333   22.77181  2.69338801 0.194196739
shale:valley - sandstone:valley = 0      124.000000   22.77181  5.44532793 0.001034456
shale:north - shale:hilltop = 0           48.666667   22.77181  2.13714483 0.433310219
shale:south - shale:hilltop = 0           52.666667   22.77181  2.31280057 0.343872870
shale:valley - shale:hilltop = 0         115.333333   22.77181  5.06474050 0.002235771
shale:south - shale:north = 0              4.000000   22.77181  0.17565574 0.999999580
shale:valley - shale:north = 0            66.666667   22.77181  2.92759566 0.130670832
shale:valley - shale:south = 0            62.666667   22.77181  2.75193992 0.176254471
```

```
> glhtSig(spint.mc)
```

```
[1] "shale:valley - sandstone:hilltop" "shale:valley - sandstone:north"
[3] "shale:valley - sandstone:south"   "shale:valley - sandstone:valley"
[5] "shale:valley - shale:hilltop"
```

```
> cld(spint.mc)
```

```
sandstone:hilltop    sandstone:north    sandstone:south   sandstone:valley      shale:hilltop
              "a"                "a"                "a"                "a"                "a"
      shale:north        shale:south       shale:valley
             "ab"               "ab"                "b"
```

```
> fitPlot(lm1,change.order=TRUE,interval=FALSE,main="",ylim=c(20,160),
          ylab="Mean Phosphorous Level",xlab="Topographic Location",legend="topleft")
> addSigLetters(lm1,change.order=TRUE,lets=c("a","a","a","ab","a","ab","a","b"),
                pos=c(1,3,1,3,1,1,3,1))
```