

# Two-Way ANOVA

R Handout

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## Initialization

```
> library(NCStats)
> library(multcomp)      # glht()
```

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## Bacteria Example

### Background

What is the optimal temperature (27,35,43°C) 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 was recorded in millions CFU/mL (CFU=Colony Forming Units).

```
> 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$ftemp <- factor(bact$temp)
> bact$fconc <- factor(bact$conc)
> str(bact)
```

```
'data.frame':  30 obs. of  5 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 ...
 $ ftemp: Factor w/ 3 levels "27","35","43": 1 1 1 1 1 2 2 2 2 2 ...
 $ fconc: Factor w/ 5 levels "0.6","0.8","1",...: 1 2 3 4 5 1 2 3 4 5 ...
```

### Initial Summaries

```
> sumTable(cells~ftemp*fconc,data=bact,FUN=length)
```

```
      0.6 0.8 1 1.2 1.4
27    2   2 2   2   2
35    2   2 2   2   2
43    2   2 2   2   2
```

```
> sumTable(cells~ftemp*fconc,data=bact,FUN=mean,digits=0)
```

```
      0.6 0.8   1 1.2 1.4
27 102 106 160 267 131
35   88 161 170 230 198
43  134 166 136 208 164
```

```
> sumTable(cells~ftemp*fconc,data=bact,FUN=sd,digits=1)
```

```
      0.6   0.8     1   1.2   1.4
27 67.2 20.5 37.5   9.9 28.3
35   8.5   7.1 13.4   9.2 29.0
43 26.9 28.3   0.7 27.6 27.6
```

## Model Fitting and Summary

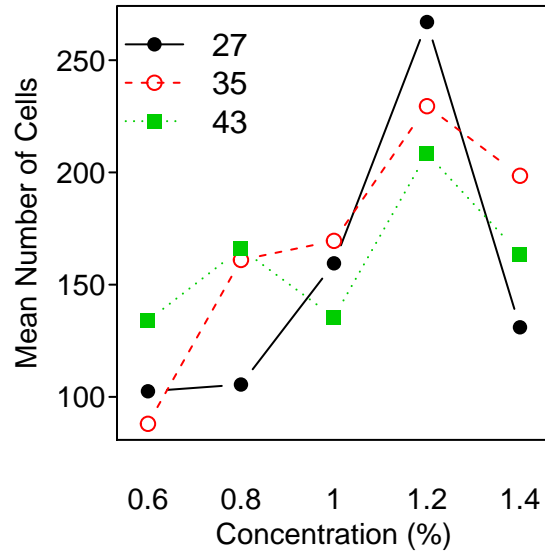
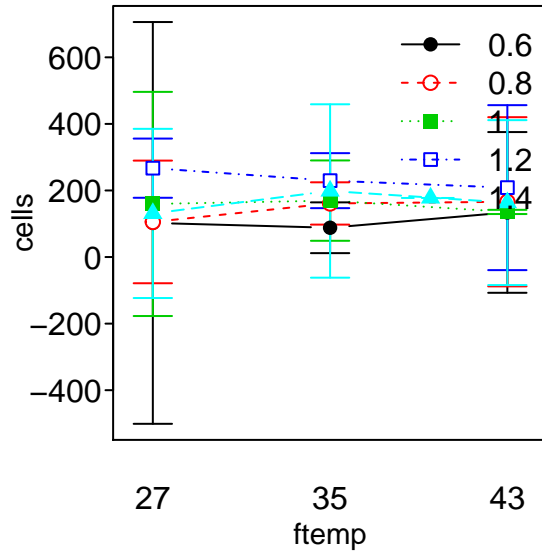
```
> lm1 <- lm(cells~ftemp*fconc,data=bact)
> anova(lm1)
```

Analysis of Variance Table

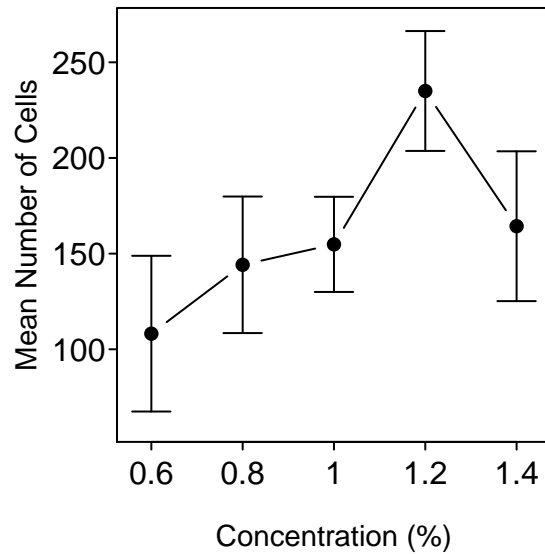
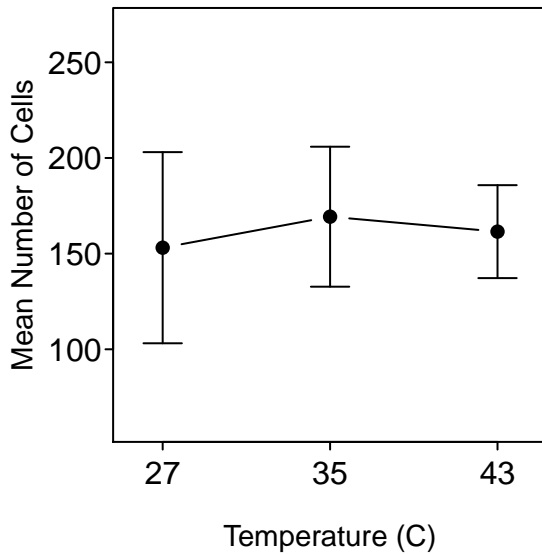
Response: cells

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

```
> fitPlot(lm1, main="") # left
> fitPlot(lm1,interval=FALSE,change.order=TRUE,xlab="Concentration (%)",
          ylab="Mean Number of Cells",legend="topleft", main="") # right
```



```
> fitPlot(lm1, which="ftemp", ylim=c(60, 270), xlab="Temperature (C)",
  ylab="Mean Number of Cells", main="") # left
> fitPlot(lm1, which="fconc", ylim=c(60, 270), xlab="Concentration (%)",
  ylab="Mean Number of Cells", main="") # right
```



## Multiple Comparisons

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

Warning in mcp2matrix(model, linfct = linfct): covariate interactions found -- default contrast might be inappropriate

```
> summary(bact.mc1)
```

### Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

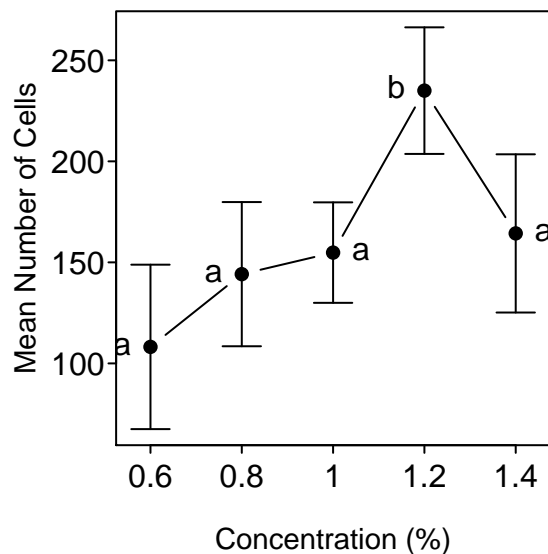
Fit: `lm(formula = cells ~ ftemp * fconc, data = bact)`

Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(> t )
0.8 - 0.6 == 0	3.0	27.7	0.108	0.999965
1 - 0.6 == 0	57.0	27.7	2.058	0.287150
1.2 - 0.6 == 0	164.5	27.7	5.939	0.000232
1.4 - 0.6 == 0	28.5	27.7	1.029	0.838173
1 - 0.8 == 0	54.0	27.7	1.950	0.334954
1.2 - 0.8 == 0	161.5	27.7	5.831	0.000280
1.4 - 0.8 == 0	25.5	27.7	0.921	0.884520
1.2 - 1 == 0	107.5	27.7	3.881	0.010985
1.4 - 1 == 0	-28.5	27.7	-1.029	0.838188
1.4 - 1.2 == 0	-136.0	27.7	-4.910	0.001503

(Adjusted p values reported -- single-step method)

```
> fitPlot(lm1,which="fconc",xlab="Concentration (%)",ylab="Mean Number of Cells",main="")  
> addSigLetters(lm1,which="fconc",lets=c("a","a","a","b","a"),pos=c(2,2,4,2,4))
```



# Soil Phosphorous Example

## Background

Soil phosphorous is important for the invasion of native vegetation by exotic weeds. Clements (1983) studied the soil phosphorous in the Sydney region (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. 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 m<sup>2</sup> 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)
```

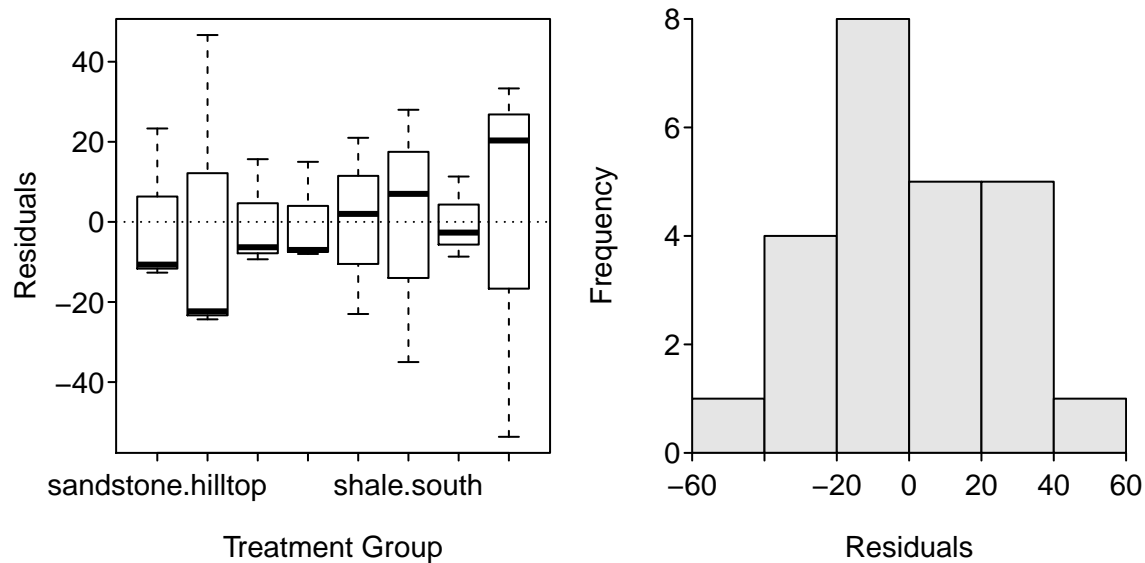
```
'data.frame':  24 obs. of  3 variables:
 $ soil: Factor w/ 2 levels "sandstone","shale": 2 2 2 2 2 2 2 2 2 2 ...
 $ topo: Factor w/ 4 levels "hilltop","north",...: 4 4 4 2 2 2 3 3 3 1 ...
 $ phos: int  98 172 185 78 77 100 117 54 96 83 ...
```

## Analysis

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

```
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group  7  0.3741 0.9043
      16
```

```
> residPlot(lm1)
```



```
> adTest(lm1$residuals)
```

Anderson-Darling normality test with x  
A = 0.2126, p-value = 0.8351

```
> outlierTest(lm1)
```

No Studentized residuals with Bonferonni  $p < 0.05$   
Largest |rstudent|:  
rstudent unadjusted p-value Bonferonni p  
1 -2.824098 0.012821 0.30769

```
> anova(lm1)
```

Analysis of Variance Table

Response: phos

	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
> view(sp)
```

	soil	topo	phos	comb
6	shale	north	100	shale:north
9	shale	south	96	shale:south
10	shale	hilltop	83	shale:hilltop
11	shale	hilltop	12	shale:hilltop
19	sandstone	south	28	sandstone:south
23	sandstone	hilltop	21	sandstone:hilltop

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

#### Analysis of Variance Table

Response: phos

	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)
```

#### Simultaneous Tests for General Linear Hypotheses

##### Multiple Comparisons of Means: Tukey Contrasts

Fit: lm(formula = phos ~ comb, data = sp)

##### Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(> t )
sandstone:north - sandstone:hilltop == 0	1.667	22.772	0.073	1.00000
sandstone:south - sandstone:hilltop == 0	19.333	22.772	0.849	0.98685
sandstone:valley - sandstone:hilltop == 0	-4.000	22.772	-0.176	1.00000
shale:hilltop - sandstone:hilltop == 0	4.667	22.772	0.205	1.00000
shale:north - sandstone:hilltop == 0	53.333	22.772	2.342	0.33052
shale:south - sandstone:hilltop == 0	57.333	22.772	2.518	0.25634
shale:valley - sandstone:hilltop == 0	120.000	22.772	5.270	0.00143
sandstone:south - sandstone:north == 0	17.667	22.772	0.776	0.99218
sandstone:valley - sandstone:north == 0	-5.667	22.772	-0.249	1.00000
shale:hilltop - sandstone:north == 0	3.000	22.772	0.132	1.00000
shale:north - sandstone:north == 0	51.667	22.772	2.269	0.36567
shale:south - sandstone:north == 0	55.667	22.772	2.445	0.28549
shale:valley - sandstone:north == 0	118.333	22.772	5.196	0.00174
sandstone:valley - sandstone:south == 0	-23.333	22.772	-1.025	0.96342
shale:hilltop - sandstone:south == 0	-14.667	22.772	-0.644	0.99746
shale:north - sandstone:south == 0	34.000	22.772	1.493	0.80049
shale:south - sandstone:south == 0	38.000	22.772	1.669	0.70569
shale:valley - sandstone:south == 0	100.667	22.772	4.421	0.00789
shale:hilltop - sandstone:valley == 0	8.667	22.772	0.381	0.99992
shale:north - sandstone:valley == 0	57.333	22.772	2.518	0.25570
shale:south - sandstone:valley == 0	61.333	22.772	2.693	0.19380
shale:valley - sandstone:valley == 0	124.000	22.772	5.445	< 0.001
shale:north - shale:hilltop == 0	48.667	22.772	2.137	0.43410
shale:south - shale:hilltop == 0	52.667	22.772	2.313	0.34387
shale:valley - shale:hilltop == 0	115.333	22.772	5.065	0.00233
shale:south - shale:north == 0	4.000	22.772	0.176	1.00000
shale:valley - shale:north == 0	66.667	22.772	2.928	0.13105
shale:valley - shale:south == 0	62.667	22.772	2.752	0.17590

(Adjusted p values reported -- single-step method)

```
> 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"
```

```
> 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))
```

