Experimental Design and Statistical Methods

Workshop

COMPARISON OF MEANS OF SEVERAL RANDOM SAMPLES. ANOVA

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UAB

Items

- One-way ANOVA (completely random design):
  - Principles for partitioning variation
  - ANOVA table and F test
  - Conditions of applicability
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    - Equality of variances
  - Comparisons of means
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- Basic commands
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  - tapply - cbind
  - aov – anova, summary lm
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  - contrasts
- Libraries
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  - agricolae

Inferences on more than two samples

This session is devoted to present methods to compare location parameters (means) of more than two samples through parametric procedures.

When we have more than two samples, **ANOVA techniques are preferable over several pair-wise comparisons** because of two main reasons:

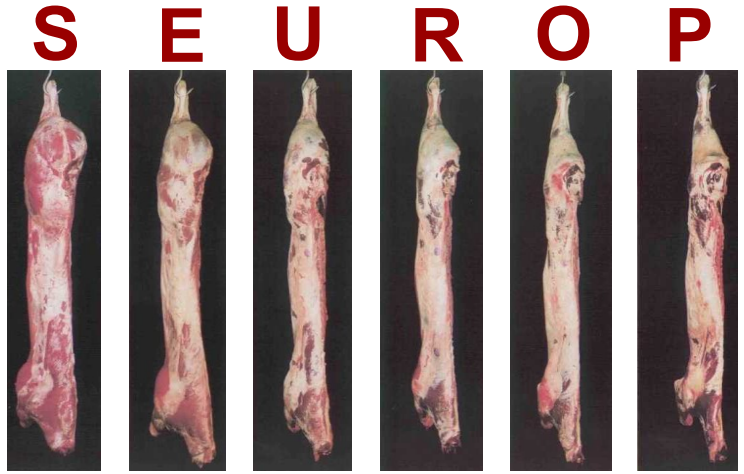
1. **We get a better estimate of the within group (residual) variance**.
2. **The probability of false positives (Type I errors) is lower**. The *Experiment-wise error rate*, i.e., the probability of finding a significant result by chance when c comparisons are made is $1-(1-\alpha)^c$, α being the *Comparison-wise error rate*.

Comparisons	Prob. Not Significant	Prob. Significant
1	$(1-\alpha)$	$1-(1-\alpha)=\alpha$
2	$(1-\alpha)^2$	$1-(1-\alpha)^2$
.	.	.
.	.	.
c	$(1-\alpha)^c$	$1-(1-\alpha)^c$

One-way ANOVA (1) – Completely Random Design

Suppose we have 30 young bulls assigned randomly to three treatments (feed additives) and we measure carcass conformation on a SEUROP scale.

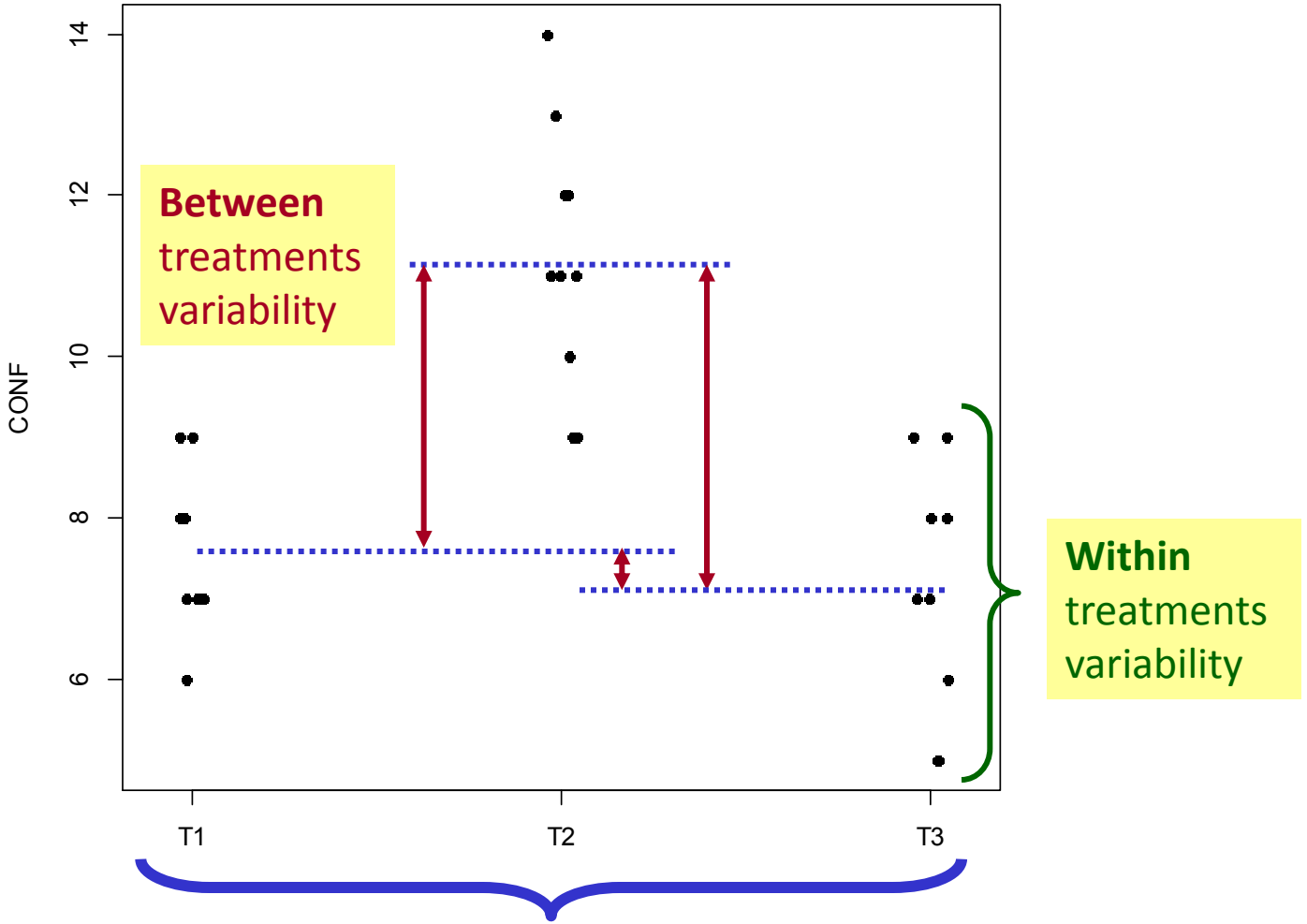
τ_i	T1	T2	T3
	7	9	8
	8	13	7
	9	12	8
	7	11	5
	6	14	6
	9	11	9
	8	10	8
y_{ij}	7	12	5
	8	9	7
	7	11	9



with intermediate scores (+/-)

¿Are significantly different the means of the three treatments, $H_0 : \bar{y}_1 = \bar{y}_2 = \bar{y}_3$ **?**

One-way ANOVA (2)



One-way ANOVA (3)

Model:

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where i) $1 \rightarrow t$
j) $1 \rightarrow n$, balanced design
$1 \rightarrow n_i$, unbalanced design

Level of a factor (fixed effect)

Mean of level i — Overall mean

$$y_{ij} - \bar{y}_{..} = (y_{ij} - \bar{y}_{i.}) + (\bar{y}_{i.} - \bar{y}_{..})$$

Total SS, SS_T

Within groups (residual), SS_W

Between groups, SS_B

Sums of squares (*SS*)

By squaring and summing these quantities, we arrive after some algebra at the computing formulas of the next slide

¡We make a partition of the variation!

One-way ANOVA (4) (for a balanced design)

Source of variation	Degrees of freedom	Sum of squares	Mean squares	*F*
Between groups	$t-1$	SS_B	$SS_B/(t-1)$	MS_B/MS_W
Within groups (error)	$t(n-1)$	SS_W	$SS_W/(tn-t)$	
Total	$tn-1$	SS_T	$SS_T/(tn-1)$	

$$F = \frac{\left(SS_B/\sigma^2\right)/(t-1)}{\left(SS_W/\sigma^2\right)/(tn-t)}$$

$$SS_B = \frac{\sum_i y_{i.}^2}{n} - \frac{y_{..}^2}{tn} \qquad SS_W = \sum_{ij} y_{ij}^2 - \frac{\sum_i y_{i.}^2}{n}$$

$$SS_T = SS_B + SS_W \Rightarrow SS_T = \sum_{ij} y_{ij}^2 - \boxed{\frac{y_{..}^2}{tn}}$$

Correction term

One-way ANOVA (5), contrast of hypothesis

The **null hypothesis** and the **alternative hypothesis** can be stated as:

H_0: $\mu_1 = \mu_2 = \mu_3$, the population means are equal

H_1: $\mu_i \neq \mu_{i'}$, for at least one pair (i, i'), the means are not equal

An **equivalent formulation** of the hypothesis is:

H_0: $\tau_1 = \tau_2 = \tau_3$, there is no difference among treatments

H_1: $\tau_i \neq \tau_{i'}$, for at least one pair (i, i'), a difference among treatments exist

It can be shown that the **expectations of the mean squares** are:

$E(MS_W) = \sigma^2$ ➡ MS_W is an unbiased estimator of σ^2

$$E(MS_B)\begin{cases} = \sigma^2 \text{ if } H_0 \\ > \sigma^2 \text{ if not } H_0 \end{cases} \Rightarrow \quad E(MS_B) = \sigma^2 + \frac{n\sum_i \tau_i^2}{t-1}, \quad \text{for} \sum_i \tau_i = 0$$

$$F = \frac{E(MS_B)}{E(MS_W)} = \frac{\sigma^2 + \frac{n\sum_i \tau_i^2}{t-1}}{\sigma^2} > 1 \quad \text{if not } H_0$$

One-way ANOVA (6), our example

Source of variation	Degrees of freedom	Sum of squares	Mean squares	*F*
Between groups	3 – 1 = 2	2350.4-2253.333 = **97.067**	97.067 / 2 = **48.533**	48.5333/ 1.911 = **25.40**
Within groups (error)	3*(10–1) = 27	2402 – 2350.4 = **51.6**	51.6/ 27 = **1.911**	
Total	3*10 – 1 = 29	2402 – 2253.33 = **148.667**	148.667 / 29 = **5.126**	

$$\sum_{ij} y_{ij}^2 \Rightarrow 7^2 + 8^2 + 9^2 \ldots + 5^2 + 7^2 + 9^2 = 2402$$

$$\frac{\sum_i y_{i.}^2}{n} \Rightarrow \frac{76^2 + 112^2 + 72^2}{10} = 2350.4$$

$$\frac{y_{..}^2}{tn} \Rightarrow \frac{(7+8+9+\ldots+5+7+9)^2}{3\times 10} = 2253.333$$

$25.40 > F_{2,27}$ (=3.35)

Reject H_0, means are significantly different (p<0.05), BUT which ones?

Pre-planed or Multiple comparisons

Protocol to develop ANOVA

1. Import the data (reorganize levels of the factor, if needed)
2. Assess normality/homogeneity of variance using boxplots
3. Assess homogeneity of variance assumption
4. Test H_0 that population group mean are all equal – perform analysis of variance
5. Examine the ANOVA table
6. Make diagnostic plots
7. Perform post-hoc tests
8. Make graphics
9. Compute the power of the ANOVA test

(Adapted from Logan, 2010)

Conditions of applicability of ANOVA

The conditions of applicability (additivity and normal errors identically distributed with common variance σ^2) must be met when we want to make some inference, such as the estimation of the confidence interval or some hypothesis testing. There are **two main conditions** to be checked:

1. **Normality of errors**. We check this in the residuals, our best estimate of the errors. The analysis of variance, however, is **little sensitive (robust) to the non normality** of the populations under study. In practice it is enough to avoid the use of the ANOVA when the samples deviate heavily from the normal distribution or the distribution of the samples is very different, mainly in small samples.
2. **Homogeneity of within group variances**. It can be tested through the Bartlett χ^2 test, among others. **Its importance is relatively secondary when sample sizes are equal** (balanced designs).

If the conditions of applicability are not met, we can use some **transformations**.

Testing for homogeneity of within group variances (1)

```
> bartlett.test(CONF~TRT, CONFBEEF)

        Bartlett test of homogeneity of variances

data:  CONF by TRT
Bartlett's K-squared = 2.3132, df = 2, p-value = 0.3145
```

The null hypothesis of homogeneity of within treatment variance is not rejected

Be careful:
The Bartlett test is too sensitive to the deviations to normality and may indicate non normality instead of variance heterogeneity

```
> 1-pchisq(2.3132,2)
[1] 0.3145538
```

Testing for homogeneity of within group variances (2)

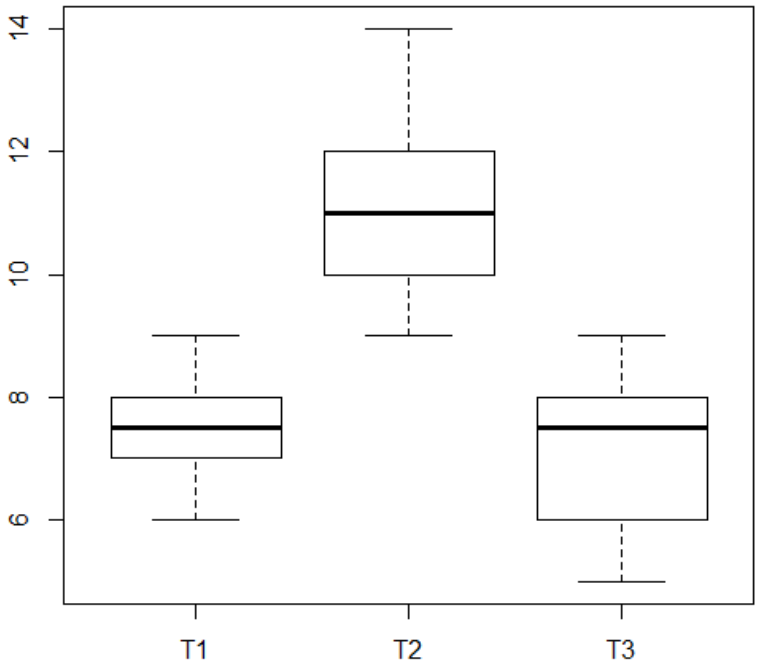
```
> #Levene test
> library(car)
> leveneTest(y = CONF, group = TRT)

Levene's Test for Homogeneity of Variance (center
= median)
      Df F value Pr(>F)
group  2   0.809 0.4558
      27
```

The null hypothesis of homogeneity of within treatment variance is not rejected

```
> 1-pf(0.809,2,27)
[1] 0.4558065
```

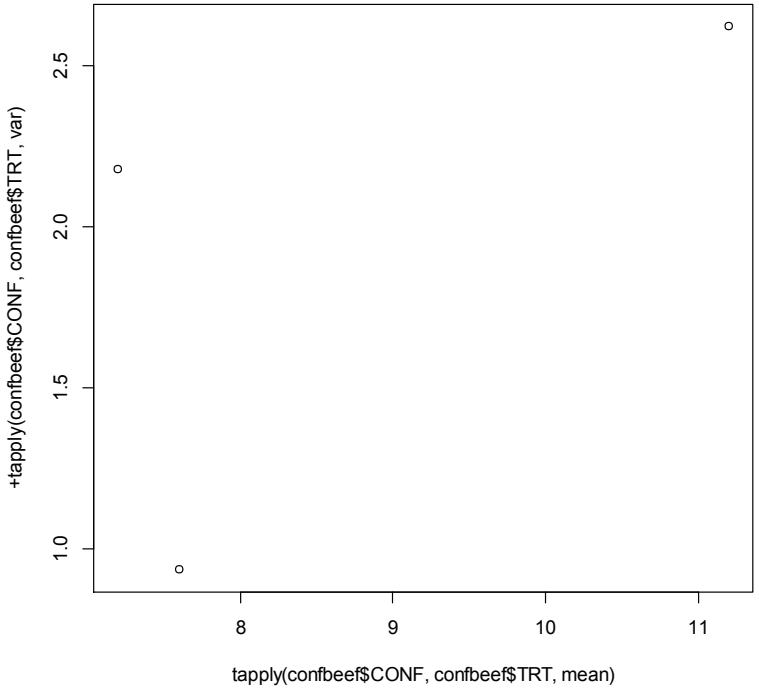
Working ANOVA with R (1)

```
> boxplot(CONF~TRT, CONFBEEF)
```



No obvious violations of normality and homogeneity of variance (boxplots not asymmetrical and do not vary greatly in size)

```
> plot(tapply(CONF, TRT, mean),
+    tapply(CONF, TRT, var))
```



No obvious relationship between group (treatment) mean and variance

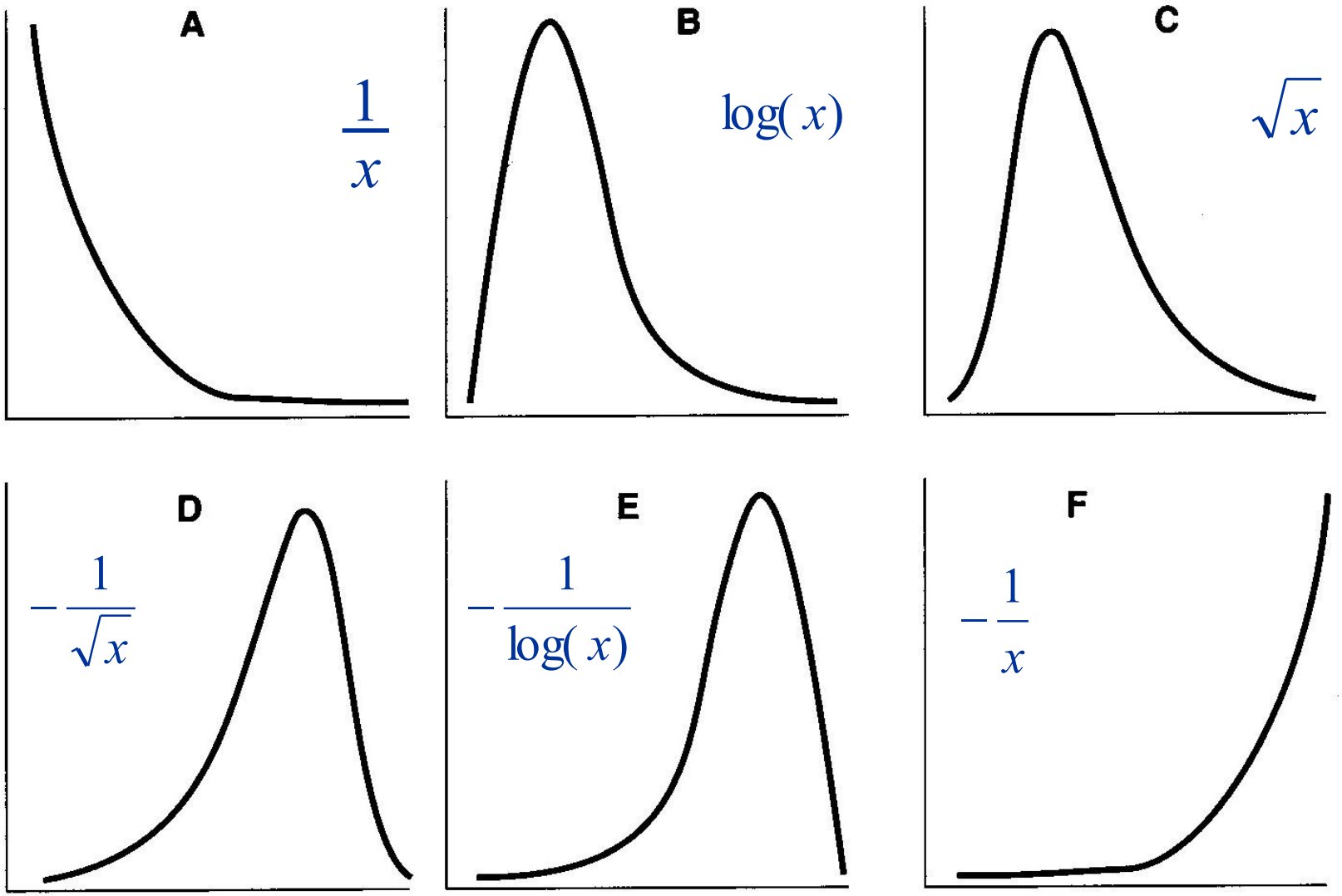
Transformations (1)

The objective is to normalise the distribution and to stabilise the variances. If this procedure does not give satisfactory results Non Parametric Tests can be used.

1. **Logarithmic**. When the treatments have a multiplicative effect, i.e., when they increment or decrement the measurements in a percent and not in a fixed quantity.
2. **Root square**. For data consisting of integers coming from counting (ticks on a cow). It tends to equalise σ^2.
3. **Angular** (arcsine). Data are the number of individuals with some particular characteristic (percentages and proportions). Equalises σ^2.
4. **Probit**. For percentage data, like mortality. It is used in pharmacology.
5. **Box-Cox**. A general methodology to transform data.

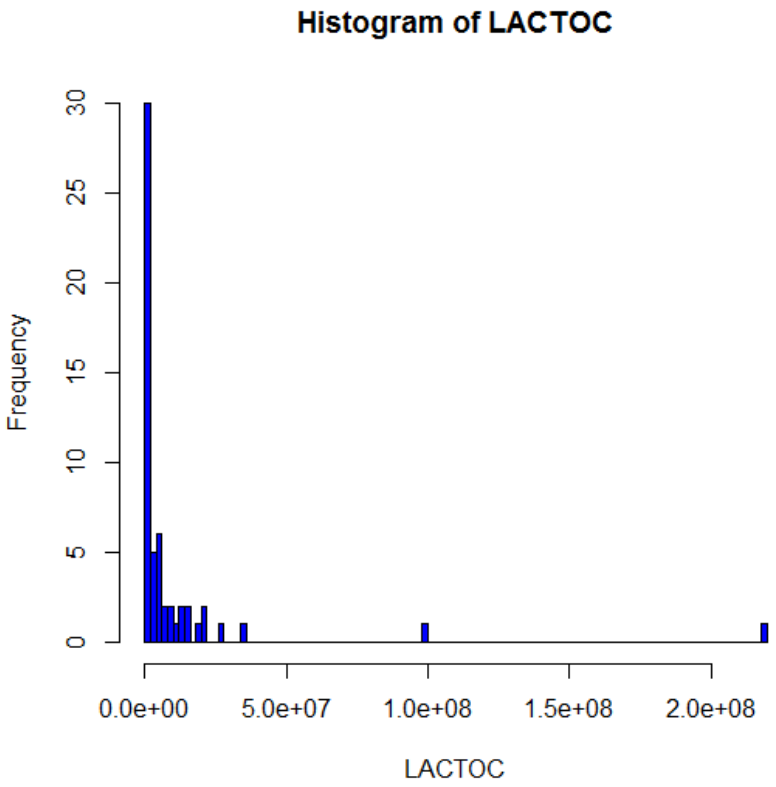
To present a true mean value of data in the linear scale it is necessary to reconvert the transformed mean. The standard deviation in this case is of no value and you should compute confidence limits of the transformed data and then convert these to the linear scale.

Transformations (2)

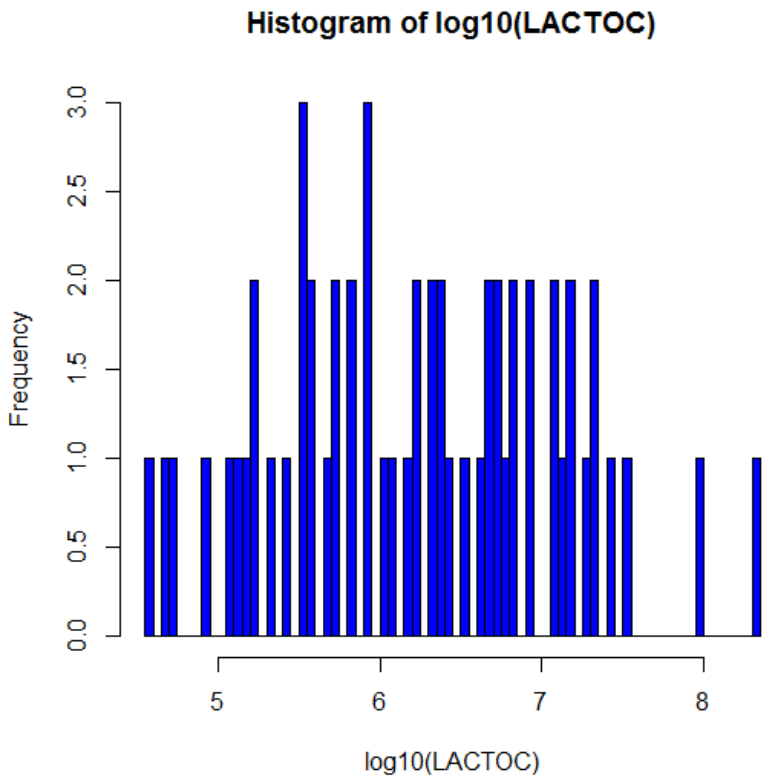


Transformations (example in microbiology)

```
>hist(LACTOC,col="blue",breaks=100)
```




```
>hist(log10(LACTOC),col="blue",
breaks=100)
```



Working ANOVA with R (2)

```
> CONFBEEF.AOV<-aov(CONF~TRT, CONFBEEF)
> anova(CONFBEEF.AOV)

Analysis of Variance Table
Response: CONF
          Df Sum Sq Mean Sq F value   Pr(>F)
TRT        2 97.067  48.533  25.395 6.25e-07 ***
Residuals 27 51.600   1.911
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
> 1-pf(25.395,2,27)
[1] 6.250862e-07
```

This is a typical ANOVA table that includes the *p*-value for the significance of the *F* value. This allows us to **reject the overall null hypothesis** of no influence of the factors included in the model. In this case we reject H_0 of equality among all treatments.

Working ANOVA with R (2b)

```
> CONFBEEF.LM<-lm(CONF~TRT, CONFBEEF)
> anova(CONFBEEF.LM)

Analysis of Variance Table

Response: CONF
          Df Sum Sq Mean Sq F value    Pr(>F)
TRT        2 97.067  48.533  25.395 6.25e-07 ***
Residuals 27 51.600   1.911
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Observe that we have used **lm** instead of **aov**. **lm** stands for Linear Model, a more general procedure than **aov**.

Working ANOVA with R (3)

```
> CONFBEEF.AOV<-aov(CONF~TRT, CONFBEEF)
> summary.lm(aov(CONF~TRT))

Call: aov(formula = CONF ~ TRT)
Residuals:
   Min     1Q Median     3Q    Max
  -2.2   -0.6   -0.2    0.8    2.8

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   7.6000     0.4372  17.385 3.44e-16 ***
TRTT2         3.6000     0.6182   5.823 3.38e-06 ***
TRTT3        -0.4000     0.6182  -0.647    0.523

Residual standard error: 1.382 on 27 degrees of freedom
Multiple R-squared: 0.6529,     Adjusted R-squared: 0.6272
F-statistic:  25.4 on 2 and 27 DF,  p-value: 6.25e-07
```

R-squared is a measure of the fit of the model, SS_{Model} / SS_T, and ranges from 0 to 1. It quantifies the proportion of (the variability) of the dependent variable explained by the model (independent variables), in this case 65.29%.

Residual standard error is an estimate of the within group standard deviation.

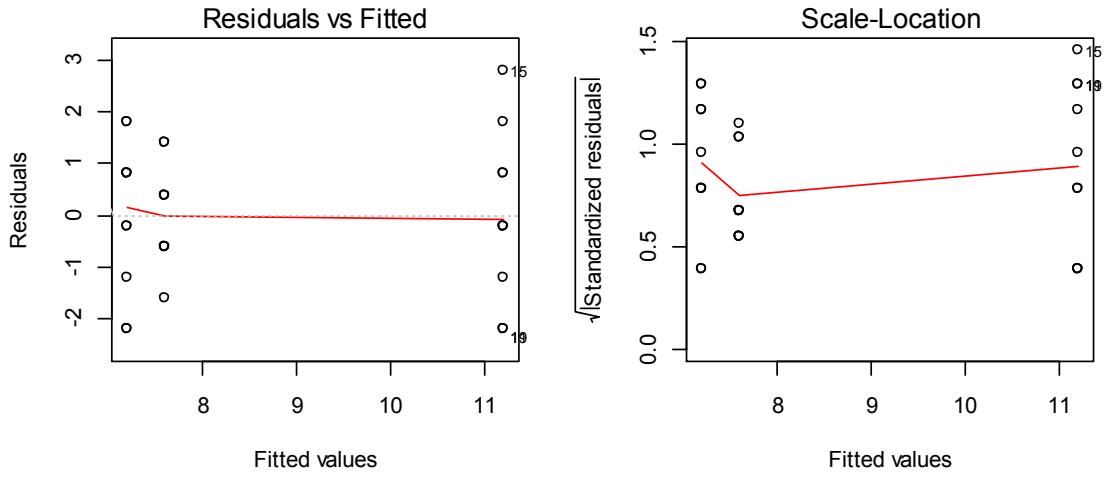
Working ANOVA with R (3b)

```
> CONFBEEF.LM<-lm(CONF~TRT, CONFBEEF)
> summary(CONFBEEF.LM)

Call:
lm(formula = CONF ~ TRT, data = CONFBEEF)

Residuals:
   Min     1Q Median     3Q    Max
  -2.2   -0.6   -0.2    0.8    2.8

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   7.6000     0.4372  17.385 3.44e-16 ***
TRTT2         3.6000     0.6182   5.823 3.38e-06 ***
TRTT3        -0.4000     0.6182  -0.647    0.523
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.382 on 27 degrees of freedom
Multiple R-squared: 0.6529,     Adjusted R-squared: 0.6272
F-statistic:  25.4 on 2 and 27 DF,  p-value: 6.25e-07
```

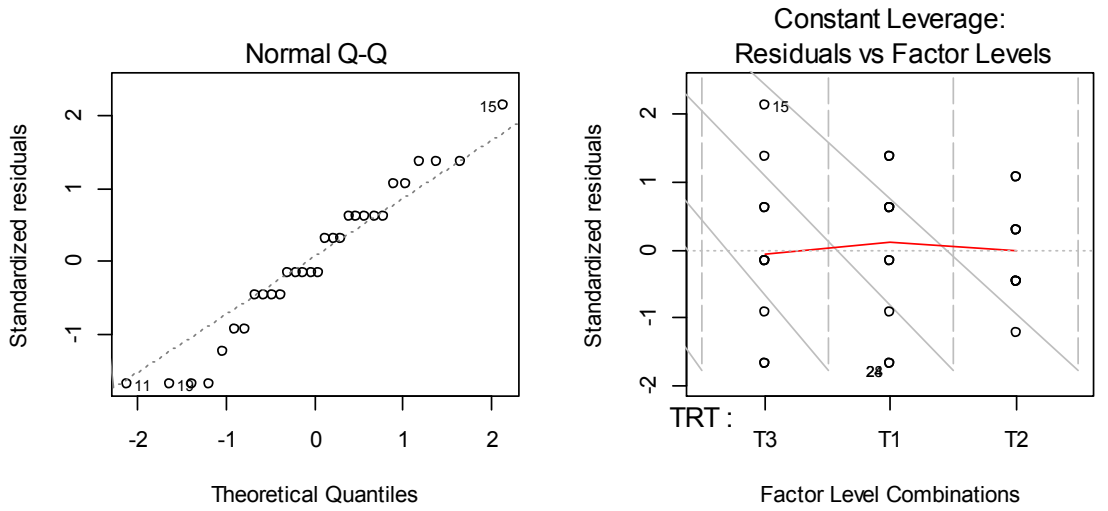
Working ANOVA with R (4). Analysis of residuals

```
> layout(matrix(c(1,2,3,4),2,2))
> plot(CONFBEEF.AOV)
```

$$\hat{e}_{ij} = y_{ij} - \hat{\mu} - \hat{\tau}_i$$



No obvious violation of homogeneity of variance: no wedge shape in residuals.



No obvious violation of normality: Q-Q plot of residuals is linear.

Cook's D values meaningless in ANOVA.

Multiple comparisons

If H_0 is not rejected, it is not necessary or appropriate to further analyse the problem, although the researcher must be aware of the possibility of a Type II error.

If H_0 is rejected, then we must question which treatment or treatments caused a differential effect, that is, between which groups is the significant difference found.

For *t* treatments, there is a total of $\binom{t}{2}$ pair-wise comparisons of means. For each comparison there is the possibility of making Type I or Type II errors.

Looking at the experiment as a whole, the probability of making an error in conclusion is defined as the Experiment-wise Error Rate (EER).

There are many procedures of pair-wise comparisons of means: Bonferroni, Duncan, Dunnet, LSD, Scheffé, Student-Newman-Keuls, **Tukey**, among others.

Working ANOVA with R (5)

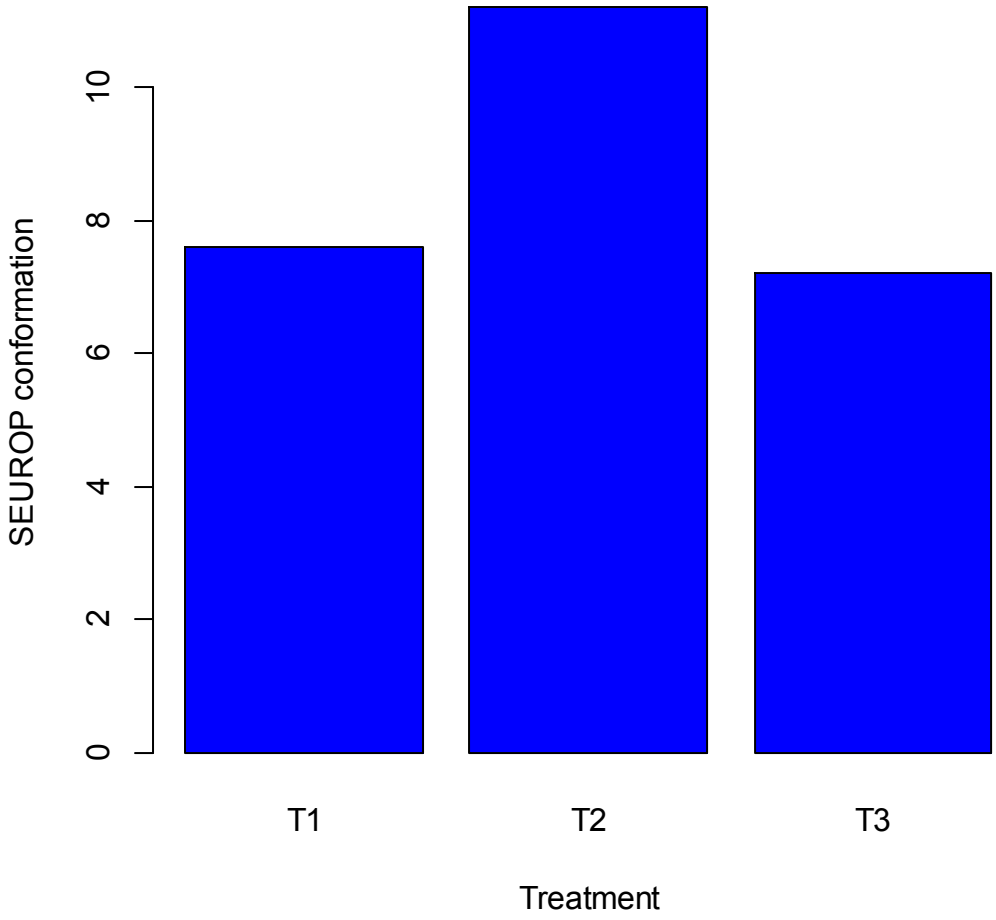
Before comparing the means, we may want to print a table with them:

```
> M<-tapply(CONF, TRT, length)
> P<-tapply(CONF, TRT, mean)
> R<-tapply(CONF, TRT, sd)
> cbind(N=M, Mean=P, Std.dev=R)
```

```
    N Mean   Std.dev
T1 10  7.6 0.9660918
T2 10 11.2 1.6193277
T3 10  7.2 1.4757296
```

Working ANOVA with R (5b)

```
> barplot(P, xlab="Treatment", ylab="SEUROP conformation",
+ col="blue")
```



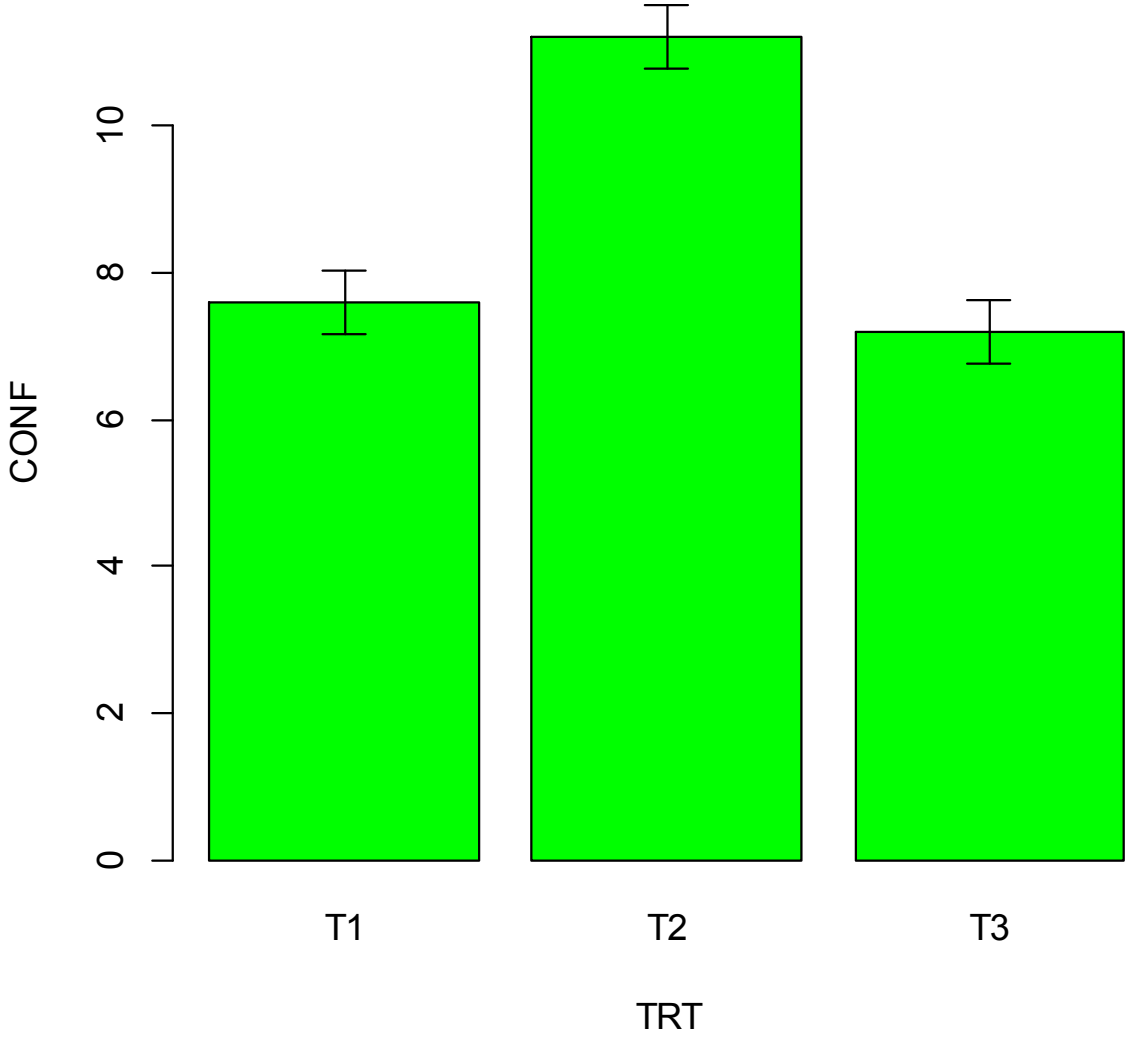
Working ANOVA with R (5c)

A function to include SE in the graphic (balanced design)

```
#Standard errors in a graphic (seBars function) from R-book p 216

seBars<-function(x,y){
         model<-lm(y~factor(x))
         reps<-length(y)/length(levels(x))
         sem<-summary(model)$sigma/sqrt(reps)
         m<-as.vector(tapply(y,x,mean))
         upper<-max(m)+sem
         nn<-as.character(levels(x))
         xs<-barplot(m,ylim=c(0,upper),names=nn,col="green",
         ylab=deparse(substitute(y)),xlab=deparse(substitute(x)))
for (i in 1:length(xs)){
         arrows(xs[i],m[i]+sem,xs[i],m[i]-sem, angle=90,code=3,length=0.1)}
}

seBars(TRT,CONF) # This executes the function for TRT and CONF
```

To get the 95% confidence intervals in the error bars write in this case **`sem*qt(.975,10)`** instead of **`sem`**

Working ANOVA with R (5d)



Working ANOVA with R (6)

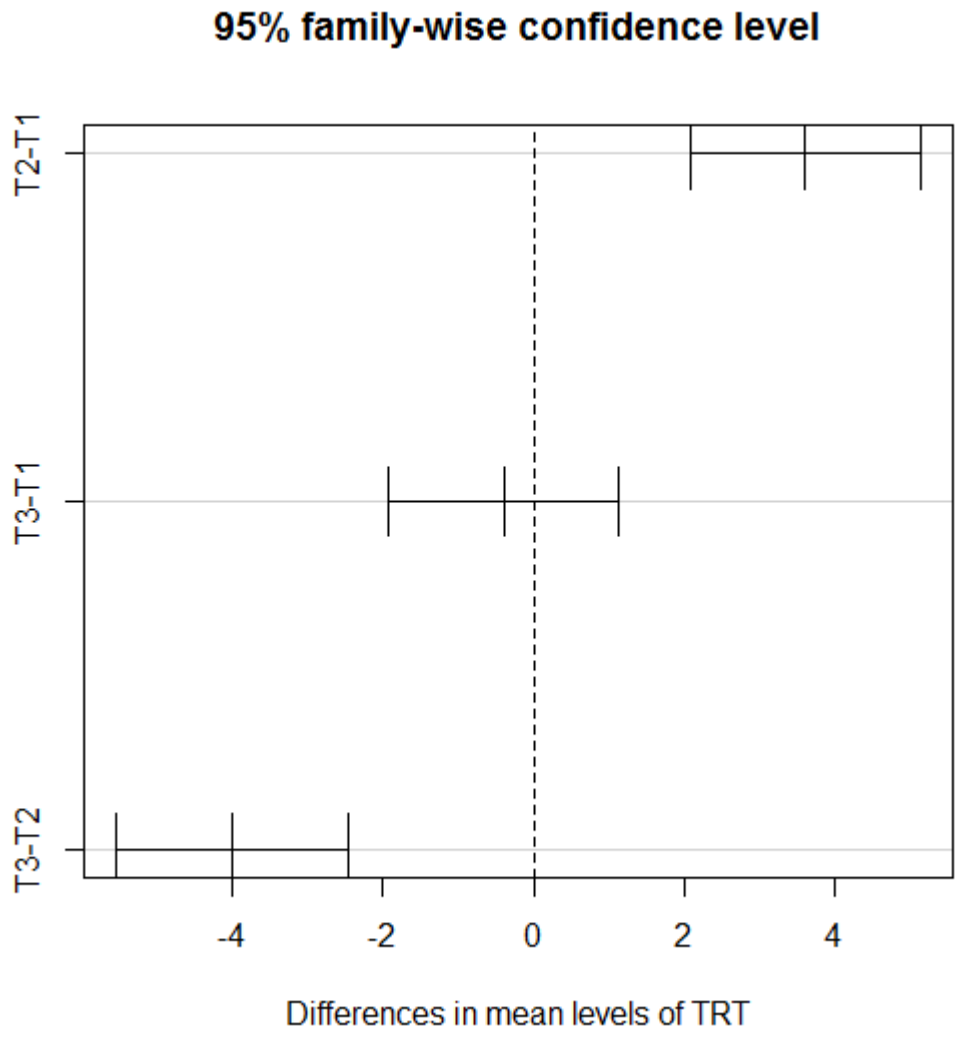
```
> CONFBEEF.TUKEY<-TukeyHSD(CONFBEEF.AOV,"TRT")
> CONFBEEF.TUKEY

  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = CONF ~ TRT, data = CONFBEEF)

$TRT
      diff       lwr       upr     p adj
T2-T1  3.6  2.067122  5.132878 0.0000099
T3-T1 -0.4 -1.932878  1.132878 0.7956248
T3-T2 -4.0 -5.532878 -2.467122 0.0000018
```

Treatment T2 differs significantly from treatments T1 and T3, which do not differ between them.

Observe that the confidence interval of the difference T3-T1 does include 0.

Working ANOVA with R (7)

```
> plot(CONFBEEF.TUKEY)
```



This is a graphic way to see the results in the previous slide.

Only the confidence interval of T3-T1 overlaps 0 (i.e., their means do not differ).

Working ANOVA with R (8)

```
> library(agricolae)
> resultHSD<-HSD.test(CONFBEEF.AOV, "TRT");resultHSD

Study:
HSD Test for CONF
Mean Square Error:  1.911111

TRT,  means
   CONF   std.err  r Min. Max.
T1  7.6 0.3055050 10    6    9
T2 11.2 0.5120764 10    9   14
T3  7.2 0.4666667 10    5    9

alpha: 0.05 ; Df Error: 27
Critical Value of Studentized Range: 3.506426
Honestly Significant Difference: 1.532878

Means with the same letter are not significantly different.
Groups, Treatments and means
a        T2      11.2
b        T1      7.6
b        T3      7.2
```

Pre-planed comparisons

Multiple comparison is the more frequent alternative. But we can be interested a priori in a limited number of comparisons.

Imagine that T2 would have been a standard diet and T1 and T3 experimental diets. We can be interested in testing if the experimental diets differ from the standard diet first, and later if differences exist between the two experimental diets.

This type of comparisons are called **contrasts**. Contrasts are linear functions of the solution's vector, in which the sum of coefficients (weighted by their sample size) must be 0. Two contrasts (c_{ij} and c_{ik}) are **orthogonal** (independent) when satisfy:

$$\sum_{i=1}^{t} n_i c_{ij} c_{ik} = 0$$

If n_i is the same for all groups, this factor can be ignored. Generally, a design with *t* levels in a factor can be partitioned to a (*t*-1) orthogonal contrasts. It can be shown that orthogonal contrasts control Type I error.

Working ANOVA with R (9)

We can use **contrasts** to test the difference between two or several groups (or combinations of them), written after the **anova** calculus.

Contrasts are linear combinations of levels that add to 1.

```
> contrasts(TRT)<-cbind(c(-0.5, 1, -0.5), c(1, 0, -1))
> round(crossprod(contrasts(TRT)), 2)
     [,1] [,2]
[1,]  1.5    0
[2,]  0.0    2
```

Independent contrasts

This tests the orthogonality of contrasts. Non diagonal elements must be 0.

```
> summary(CONFBEEF.AOV, split=list(TRT = list ("T2 vs
(T1+T3)/2" = 1, "T1 vs T3" = 2)))
                          Df Sum Sq Mean Sq F value   Pr(>F)
TRT                        2  97.07   48.53  25.395 6.25e-07 ***
  TRT: T2 vs (T1+T3)/2     1  96.27   96.27  50.372 1.25e-07 ***
  TRT: T1 vs T3            1   0.80    0.80   0.419    0.523
Residuals                 27  51.60    1.91
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

T2 is different from the mean of T1 and T3; T1 and T3 do not differ.

Power in the One-way ANOVA

The **power** of a test is the probability that a false null hypothesis is correctly rejected or a true difference is correctly declared different.

Under the H_0, the F statistic has a central F distribution with (t-1) and (tn-t = N-t) degrees of freedom. When at least one treatment effect is nonzero, the F test statistic follows a non-central F distribution with noncentrality parameter $\lambda = SS_B / MS_W$, and degrees of freedom as before. Then

$$Power = P(F > F_{\alpha,t\text{-}1,N\text{-}t} = F_{\beta})$$

using a non-central F distribution for H_1. The figure in the next slide represents the relationship between significance and power.

1. If a more stringent α is chosen (critical value shifted to the right), the power will decrease.
2. A larger SS_B and a smaller MS_W means larger λ, the noncentrality curve is shifted to the right and this augments the area under this curve (power) to the right of the critical value.

Relationship between significance and power

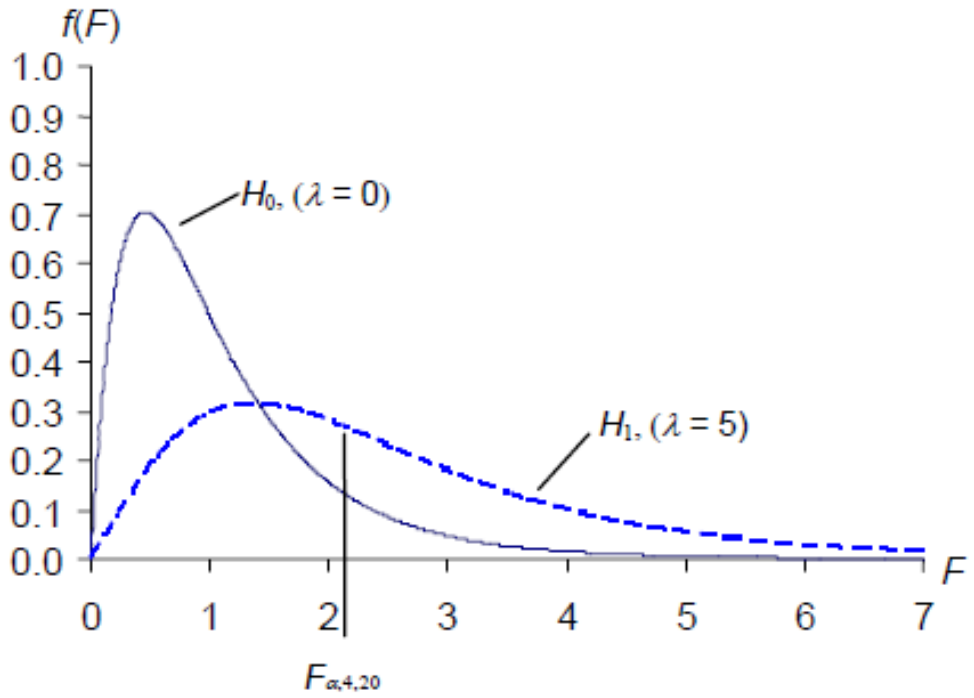


Figure 11.4 Significance and power of the F test. Under H_0 the F statistic has a central F distribution and under H_1 it has a noncentral F distribution. The distributions with 4 and 20 degrees of freedom and noncentrality parameters $\lambda = 0$ and 5 are shown. The critical value for an α level of significance is $F_{\alpha;4,20}$. The area under the H_0 curve to the right of the critical value is the level of significance (α). The area under the H_1 curve to the right of the critical value is the power ($1 - \beta$). The area under the H_1 curve on the left of the critical value is the type II error (β).

(Kaps and Lamberson, 2004)

Power of our example

Following the example of K&L, p. 229, we can write in R:

```
> #Power in a CRD-one way ANOVA
> ACONFBEEF <- anova(lm(CONF~TRT, CONFBEEF))
> DFB <- ACONFBEEF[["Df"]][1]
> DFW <- ACONFBEEF[["Df"]][2]
> SSB <- ACONFBEEF[["Sum Sq"]][1]
> MSW <- ACONFBEEF[["Mean Sq"]][2]
> LAMBDA=SSB/MSW; ALPHA=0.05
> FCRIT=qf(1-ALPHA,DFB,DFW)
> POWER=1-pf(FCRIT,DFB,DFW,LAMBDA)
> cbind(Sig.level=ALPHA, DF.between=DFB, DF.within=DFW,
 + POWER=POWER)
     Sig.level DF.between DF.within     POWER
[1,]      0.05          2        27 0.9999942
```

Observe how particular values of the ANOVA table are extracted

This value is bigger than the 0.8 usually required.

The power of the tests was very high because of the differences among means of treatments (*TRT MS*) were much higher than the differences between observations of the same group (*Residual MS*).

Power of our example (easier)

```
> power.anova.test(groups=3, n=10,
between.var=48.533, within.var=1.911,
sig.level=0.05)

Balanced one-way analysis of variance power calculation

          groups = 3
               n = 10
    between.var = 48.533
     within.var = 1.911
      sig.level = 0.05
          power = 1

 NOTE: n is number in each group
```

Sample size in a One-way ANOVA (programme)

In a previous lesson, the number of replications necessary for a test of difference between two means was given. With more than two means, the level of significance must be adjusted for multiple comparisons.

An alternative is to compute sample size from power calculations:

```
> for (n in 2:20) {
+ DFW[n] <- (DFB+1)*(n-1)
+ FCRIT[n]<-qf(1-ALPHA,DFB,DFW[n])
+ POWER[n]<-1-pf(FCRIT[n],DFB,DFW[n],LAMBDA)
+      if(POWER[n]>=.80 && n<10){
+      print(paste("n =", as.numeric(n),"  ", "Power =",
round(POWER[n], digits=7)), quote=FALSE)
+      }
+      else if(POWER[n]>=.80 && n>=10){
+      print(paste("n =", as.numeric(n)," ", "Power =",
round(POWER[n], digits=7)), quote=FALSE)
+      }
+ }
```

Sample size in a One-way ANOVA (output)

```
[1] n = 2     Power = 0.9378067
[1] n = 3     Power = 0.9984039
[1] n = 4     Power = 0.999802
[1] n = 5     Power = 0.9999425
[1] n = 6     Power = 0.9999741
[1] n = 7     Power = 0.9999851
[1] n = 8     Power = 0.9999901
[1] n = 9     Power = 0.9999927
[1] n = 10    Power = 0.9999942
[1] n = 11    Power = 0.9999953
[1] n = 12    Power = 0.999996
[1] n = 13    Power = 0.9999965
[1] n = 14    Power = 0.9999968
[1] n = 15    Power = 0.9999971
[1] n = 16    Power = 0.9999974
[1] n = 17    Power = 0.9999975
[1] n = 18    Power = 0.9999977
[1] n = 19    Power = 0.9999978
[1] n = 20    Power = 0.9999979
```

The required sample size (= 2) is actually a very low number, but it was expected if we remember that RMSE was very low and the differences among groups very high

A matrix view of ANOVA (1)

Suppose we have the following data corresponding to the birth weight (kg) of the progeny of three sires:

S1	S2	S3
45	32	35
47	40	37
46		39

$$\begin{bmatrix} 45 \\ 47 \\ 46 \\ 32 \\ 40 \\ 35 \\ 37 \\ 39 \end{bmatrix} = \begin{bmatrix} 1 & 1 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 \\ 1 & 0 & 0 & 1 \\ 1 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \mu \\ s_1 \\ s_2 \\ s_3 \end{bmatrix} + \begin{bmatrix} \varepsilon_{11} \\ \varepsilon_{12} \\ \varepsilon_{13} \\ \varepsilon_{21} \\ \varepsilon_{22} \\ \varepsilon_{31} \\ \varepsilon_{32} \\ \varepsilon_{33} \end{bmatrix}$$

Incidence or design matrix

One way ANOVA design

$$y_{ij} = \mu + s_i + \varepsilon_{ij}$$

Fixed effect

In matrix terms

$$\mathbf{y} = \mathbf{Xb} + \boldsymbol{\varepsilon}$$

Fixed model, although ε is random

We are interested in estimating the value of the elements of vector **b**

A matrix view of ANOVA (2)

Normal equations: $\mathbf{X'X}\hat{\mathbf{b}} = \mathbf{X'y} \quad \Rightarrow \quad \hat{\mathbf{b}} = (\mathbf{X'X})^{-1}\mathbf{X'y}$

$$\mathbf{X'X} = \begin{bmatrix} 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 \end{bmatrix} \begin{bmatrix} 1 & 1 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 \\ 1 & 0 & 0 & 1 \\ 1 & 0 & 0 & 1 \end{bmatrix} = \begin{bmatrix} 8 & 3 & 2 & 3 \\ 3 & 3 & 0 & 0 \\ 2 & 0 & 2 & 0 \\ 3 & 0 & 0 & 3 \end{bmatrix}$$

$$\mathbf{X'y} = \begin{bmatrix} 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 \end{bmatrix} \begin{bmatrix} 45 \\ 47 \\ 46 \\ 32 \\ 40 \\ 35 \\ 37 \\ 39 \end{bmatrix} = \begin{bmatrix} 321 \\ 138 \\ 72 \\ 111 \end{bmatrix} = \begin{bmatrix} y_{..} \\ y_{1.} \\ y_{2.} \\ y_{3.} \end{bmatrix}$$

Total number →

Obs. Sire 1 →

$$\begin{bmatrix} 8 & 3 & 2 & 3 \\ 3 & 3 & 0 & 0 \\ 2 & 0 & 2 & 0 \\ 3 & 0 & 0 & 3 \end{bmatrix} \begin{bmatrix} \hat{\mu} \\ \hat{s}_1 \\ \hat{s}_2 \\ \hat{s}_3 \end{bmatrix} = \begin{bmatrix} 321 \\ 138 \\ 72 \\ 111 \end{bmatrix} \quad \Rightarrow \quad \begin{bmatrix} \hat{\mu} \\ \hat{s}_1 \\ \hat{s}_2 \\ \hat{s}_3 \end{bmatrix} = \begin{bmatrix} 8 & 3 & 2 & 3 \\ 3 & 3 & 0 & 0 \\ 2 & 0 & 2 & 0 \\ 3 & 0 & 0 & 3 \end{bmatrix}^{-1} \begin{bmatrix} 321 \\ 138 \\ 72 \\ 111 \end{bmatrix}$$

But the determinant of $\mathbf{X'X}$ = 0: singular, it has not an inverse.

A matrix view of ANOVA (3)

A convenient generalized inverse of $\mathbf{X'X}$ is $\mathbf{G}$:

$$\mathbf{G} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 1/3 & 0 & 0 \\ 0 & 0 & 1/2 & 0 \\ 0 & 0 & 0 & 1/3 \end{bmatrix}$$

Observe that we can take the $\mathbf{A}$ square submatrix with rank = rank($\mathbf{X'X}$) from different positions of $\mathbf{X'X}$, and then put it in the corresponding position in $\mathbf{G}$. This can give different generalized inverses.

And then,

$$\begin{bmatrix} \hat{\mu} \\ \hat{s}_1 \\ \hat{s}_2 \\ \hat{s}_3 \end{bmatrix} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 1/3 & 0 & 0 \\ 0 & 0 & 1/2 & 0 \\ 0 & 0 & 0 & 1/3 \end{bmatrix} \begin{bmatrix} 321 \\ 138 \\ 72 \\ 111 \end{bmatrix} = \begin{bmatrix} 0 \\ 138/3 \\ 72/2 \\ 111/3 \end{bmatrix} \leftarrow$$

This is in fact a solution vector for $\mathbf{b}$, not an estimator of $\mathbf{b}$. The vector depends upon the generalized inverse calculated.

A matrix view of ANOVA (4)

It is possible to obtain a vector of predicted values from $\hat{\mathbf{b}}$, $\hat{\mathbf{y}} = \mathbf{X}\hat{\mathbf{b}}$. The square sum of the deviations of observed $\mathbf{y}$'s to their predicted values is the error or residual sum of squares (SS_e):

$$SS_e = \sum_i \sum_j \left(y_{ij} - \hat{y}_{ij}\right)^2 = \cdots = \mathbf{y}'\mathbf{y} - \mathbf{b}'\mathbf{X}'\mathbf{y} \quad \text{(Searle, 1982)}$$

This sum of squares is invariant to any generalized inverse that we use to estimate $\hat{\mathbf{b}}$

Furthermore, the total (corrected) sum of squares is:

$$SS_T = \sum_i \sum_j \left(y_{ij} - \bar{y}_{..}\right)^2 = \cdots = \mathbf{y}'\mathbf{y} - N\bar{y}^2$$

Then, we can write the following ANOVA table:

Source of Variation	d.f.	Sums of Squares	F
Between groups (Fitting of the model)	$t-1$	$SS_B = \hat{\mathbf{b}}'\mathbf{X}'\mathbf{y} - N\bar{y}^2$	$\frac{SS_B/(t-1)}{SS_e/(N-t)} = \frac{MS_B}{MS_e}$
Within groups (error)	$t(n-1)$	$SS_e = \mathbf{y}'\mathbf{y} - \hat{\mathbf{b}}'\mathbf{X}'\mathbf{y}$	
Total	tn-1	$SS_T = \mathbf{y}'\mathbf{y} - N\bar{y}^2$	$(N = tn)$

This is the way in which statistical packages like R work

A matrix view of ANOVA (5)

```
> birth.aov<-aov(BIRTH.W~SIRE)
> anova(birth.aov)
Analysis of Variance Table
Response: BIRTH.W
          Df Sum Sq Mean Sq F value  Pr(>F)
SIRE       2 166.88  83.438   9.933 0.01813 *
Residuals  5  42.00   8.400

> summary.lm(aov(BIRTH.W~SIRE))
Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   46.000      1.673  27.490 1.19e-06 ***
SIRES2       -10.000      2.646  -3.780   0.0129 *
SIRES3        -9.000      2.366  -3.803   0.0126 *
Residual standard error: 2.898 on 5 degrees of freedom
Multiple R-squared: 0.7989,     Adjusted R-squared: 0.7185
F-statistic: 9.933 on 2 and 5 DF,  p-value: 0.01813
```

$\hat{\mathbf{b}} = \begin{bmatrix} 46.000 \\ -10.000 \\ -9.000 \end{bmatrix}$

Observe that the solutions for $\mathbf{b}$ in this table are different from the solutions obtained with our generalized inverse. R equals to 0 the first level of each factor.

References

Crawley M.J. 2013. *The R book*, 2nd ed. Wiley, New York.

Kaps M., LambersonW.R. 2004. *Biostatistics for the Animal Sciences*. CABI, Wallinford.

Logan M. 2010. *Biostatistical Design and Analysis Using R*. Wiley-Blackwell, Chichester.