

Analysis of Variance

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A motivating study ...

- **Suppose that we are interested in the association between a gene polymorphism and glucose levels**
- **The response variable (outcome): glucose**
- **“Risk factor”: the gene polymorphism, 6 genotypes: A, B, C, D, E, and F**
- **What can/should we do?**

A motivating study ...

- Measure glucose levels in all individuals
- Classify the individuals into 6 genotypic groups
- Compare glucose levels among the 6 groups by unpaired t-test
- There would be $2^6 = 64$ possible tests of hypothesis!
- The chance of finding at least one significant difference is $1 - (1 - 0.05)^{64} = 0.962$

A motivating study ...

- It would be nice to have one “catch all” test
 - Something which would tell us whether there is ANY difference among the 6 groups
 - If there is, we could then perform group to group comparisons to look for specific differences
 - Extension of the t-test

ANOVA: an extension of t-test

- Developed by Ronald A. Fisher, a brilliant statistician, geneticist, philosopher in the 1920s
- T-test compares means of two groups
- ANOVA compares means among *more than 2 groups*

ANOVA framework

- General idea behind ANOVA, comparing means of k groups ($k > 2$):

Null hypothesis $H_0: \mu_1 = \mu_2 = \dots = \mu_k$

Alternative hypothesis H_a : at least one difference

- P value from ANOVA answers the following question:
“Is there **ANY** difference in the means among the groups?”

Logics of ANOVA

- For a set of n values of X_i (X_1, X_2, X_3, \dots) a **deviate** is the difference between an individual value and the mean:

$$D = X_i - M$$

- A square deviate is the square of that quantity:

$$D^2 = (X_i - M)^2$$

- The sum of squared deviates is the sum of all the squared deviated:

$$\begin{aligned} SS &= (X_1 - M)^2 + (X_2 - M)^2 + (X_3 - M)^2 + \dots + (X_n - M)^2 \\ &= \sum_{i=1}^n (X_i - M)^2 \end{aligned}$$

Rationale behind ANOVA

| Group 1 | Group 2 | Group 3 | Group k |
|----------|----------|----------|----------|
| X_{11} | X_{21} | X_{31} | X_{k1} |
| X_{12} | X_{22} | X_{32} | X_{k2} |
| X_{13} | X_{23} | X_{33} | X_{k3} |
| X_{14} | X_{24} | X_{34} | X_{k4} |
| X_{15} | X_{25} | X_{35} | X_{k5} |
| X_{16} | X_{26} | X_{36} | X_{k6} |
| M_1 | M_2 | M_3 | M_k |

▲

- The variation in the sample means *between* groups is compared to the variation *within* a group.
- If the between group variation is a lot larger than the within group variation, that suggests there are some differences among the populations.

Between-group and within-group variations

A simulated example

| A | B | C |
|----|----|----|
| 40 | 40 | 40 |
| 40 | 40 | 40 |
| 40 | 40 | 40 |

- Three groups of patients; each group has 3 patients.
- Let the individual values be X_{ij} , where $i = A, B, C$ and $j = 1, 2, 3$.
- There are no differences between or within samples:

$$X_{ij} = M$$

where $M = 40$.

A simulated example

| A | B | C |
|------|------|------|
| 40-2 | 40+6 | 40-4 |
| 40-2 | 40+6 | 40-4 |
| 40-2 | 40+6 | 40-4 |



| A | B | C |
|----|----|----|
| 38 | 46 | 36 |
| 38 | 46 | 36 |
| 38 | 46 | 36 |

- There are differences **between groups**, but no differences within group.
- The model is now:

$$X_{ij} = M + \alpha_j$$

where $M = 40$; $\alpha_1 = -2$, $\alpha_2 = 6$ and $\alpha_3 = -4$.

- Note that $\alpha_1 + \alpha_2 + \alpha_3 = 0$

A simulated example

| A | B | C |
|--------|--------|--------|
| 40-2+5 | 40+6-5 | 40-4+3 |
| 40-2+2 | 40+6+1 | 40-4-2 |
| 40-2-3 | 40+6+8 | 40-4+1 |



| A | B | C |
|--------------------|------|------|
| 43 | 41 | 39 |
| 40 | 47 | 34 |
| 35 | 54 | 37 |
| 39.3 | 47.3 | 36.7 |
| overall mean: 41.1 | | |

- In reality, there is always variation in a population, so that there is sampling error.
- The model now includes an error term:

$$X_{ij} = \mu + \alpha_j + e_{ij}$$

- Effect of
treatment A: $39.3 - 41.1 = -1.8$
treatment B: $47.3 - 41.1 = 5.8$
treatment C: $36.7 - 41.1 = -4.4$

ANOVA: model and assumption

- The model:

$$Y_{ij} = \mu + \alpha_j + \varepsilon_{ij}$$

- Assumptions:

Normality

Independence

Homogeneity

- $\text{Var}(Y) = \text{Var}(M) + \text{Var}(\alpha) + \text{Var}(\varepsilon)$
= $\text{Var}(\alpha) + \text{Var}(\varepsilon)$
= Between-group + Within-group

Between-group variation

| | A | B | C |
|---------------|------|------|------|
| | 43 | 41 | 39 |
| | 40 | 47 | 34 |
| | 35 | 54 | 37 |
| Mean | 39.3 | 47.3 | 36.7 |
| Overall mean: | 41.1 | | |

- **The sum of squares for difference between groups:**
 $(39.3 - 41.1)^2 + (47.3 - 41.1)^2 + (36.7 - 41.1)^2 = 61.04$
- **But the mean of each group is calculated from 3 observations. So the “true” sum of squares is:**
 $SSB = 3*(39.3 - 41.1)^2 + 3*(47.3 - 41.1)^2 + 3*(36.7 - 41.1)^2 = 184.8$
- **Degrees of freedom: (3 groups – 1) = 2.**

Within-group variation

| | A | B | C |
|------|----------|----------|----------|
| | 43 | 41 | 39 |
| | 40 | 47 | 34 |
| | 35 | 54 | 37 |
| Mean | 39.3 | 47.3 | 36.7 |

- **SS for group A: $SS_A = (43 - 39.3)^2 + (40 - 39.3)^2 + (35 - 39.3)^2 = 32.7$**
- **SS for group B: $SS_B = (41 - 47.3)^2 + (47 - 47.3)^2 + (54 - 47.3)^2 = 84.7$**
- **SS for group C: $SS_C = (39 - 36.7)^2 + (34 - 36.7)^2 + (37 - 36.7)^2 = 12.7$**
- **SS for within group: $SSW = SS_A + SS_B + SS_C = 130.0$**
- **Degrees of freedom: $(3 - 1) + (3 - 1) + (3 - 1) = 6$**

ANOVA by R

| A | B | C |
|----|----|----|
| 43 | 41 | 39 |
| 40 | 47 | 34 |
| 35 | 54 | 37 |

```
group <- c(1,1,1,2,2,2,3,3,3)
```

```
y <- c(43, 40, 35, 41, 47, 54, 39, 34, 37)
```

```
group <- as.factor(group)
```

```
analysis <- lm(y ~ group)
```

```
summary(analysis)
```

```
anova(analysis)
```

Summary of Variation

```
> anova(analysis)
```

```
Response: y
```

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|-----------|----|---------|---------|---------|-----------|
| group | 2 | 184.889 | 92.444 | 4.2667 | 0.07037 . |
| Residuals | 6 | 130.000 | 21.667 | | |

```
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Estimate of Treatment Effects

```
> summary(analysis)
```

```
...
```

```
Coefficients:
```

| | Estimate | Std. Error | t value | Pr(> t) | |
|-------------|----------|------------|---------|----------|-----|
| (Intercept) | 39.333 | 2.687 | 14.636 | 6.39e-06 | *** |
| group2 | 8.000 | 3.801 | 2.105 | 0.080 | . |
| group3 | -2.667 | 3.801 | -0.702 | 0.509 | |

```
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 4.655 on 6 degrees of freedom
```

```
Multiple R-Squared: 0.5872,    Adjusted R-squared: 0.4495
```

```
F-statistic: 4.267 on 2 and 6 DF,  p-value: 0.07037
```

Summary of ANOVA

| Source of variation | Df | SS (sum of squares) | MS (mean squares) | F test |
|---------------------|----|---------------------|-------------------|--------|
| Between group | 2 | 184.8 | 92.4 | 4.27 |
| Within group | 6 | 130.0 | 21.7 | |
| Total | 8 | 314.8 | | |

- **F (Fisher' s) test**

$$F = MSB / MSW = 92.4 / 21.7 = 4.27$$

- Under the null hypothesis (if H_0 is true), and if $\alpha = 0.95$, the expected value of F (2, 6) is 5.14.
- Since $F < 5.14$, we conclude that there is **NO** significant difference between group means.

ANOVA using summary data

ANOVA using summary data

Galactose levels in 3 groups of patient

| Crohn disease | Colitis | Control | |
|----------------------|----------------|----------------|-------|
| 1.343 | 1.264 | 1.809 | 2.850 |
| 1.393 | 1.314 | 1.926 | 2.964 |
| 1.420 | 1.399 | 2.283 | 2.973 |
| 1.641 | 1.605 | 2.384 | 3.171 |
| 1.897 | 2.385 | 2.447 | 3.257 |
| 2.160 | 2.511 | 2.479 | 3.271 |
| 2.169 | 2.514 | 2.495 | 3.288 |
| 2.279 | 2.767 | 2.525 | 3.358 |
| 2.890 | 2.827 | 2.541 | 3.643 |
| | 2.895 | 2.769 | 3.657 |
| | 3.011 | | |

Between-group and within-group variations

| Group | N | Mean | Variance |
|--------------|----------|-------------|-----------------|
| Crohn | 9 | 1.91 | 0.265 |
| Colitis | 11 | 2.23 | 0.473 |
| Control | 20 | 2.80 | 0.277 |
| All groups | 40 | 2.44 | |

- **Between-group variation**

$$SSB = 9(1.91 - 2.44)^2 + 11(2.23 - 2.44)^2 + 20(2.80 - 2.44)^2$$

$$SSB = 5.605$$

- **Within-group variation**

$$SSW = (9-1)(0.265) + (11-1)(0.473) + (20-1)(0.277)$$

$$SSW = 12.113$$

Summary of ANOVA

| Group | Df | SS (sum of squares) | MS (mean squares) |
|---------------|----|---------------------|-------------------|
| Between group | 2 | 5.65 | 2.825 |
| Within group | 37 | 12.113 | 0.327 |
| Total | 39 | | |

- **F (Fisher' s) test**

$$F = MSB / MSW = 2.825 / 0.327 = 8.64$$

- Under the null hypothesis (if H_0 is true), and if $\alpha = 0.95$, the expected value of F (2, 37) is 4.08.
- **Since $F > 4.08$, we conclude that there is at least one between group difference**

Which groups are different ?

Posthoc comparison

Review of the t test

$$t = \frac{\bar{X}_1 - \bar{X}_2}{s \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

s is the *common standard deviation*, n_1 and n_2 are sample size for group 1 and 2, respectively.

Methods of multiple comparisons

- **LSD (least significance difference) or Fisher's method**
- **Bonferroni's method**
- **Duncan's multiple range test**
- **Scheffé**
- **Tukey's Honest Significant Difference**
- **Dunnett's test**

Least significance difference

- **LSD is defined as:**

$$LSD = t_{\alpha/2m, n-k} \sqrt{MSW \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}$$

- **Where**

k is the number of groups

n_1 and n_2 are the number of patients in group 1 and group 2

α is the significance level (usually 0.05)

m is the number of possible comparisons

$$m = C_2^k = \frac{k!}{2!(k-2)!}$$

- **Decision rule: the difference is said to be statistically significant if**

$$\left| \bar{X}_1 - \bar{X}_2 \right| > LSD$$

Tukey' s HSD procedure

- **HSD = Honestly Significant Difference**

$$Q = \frac{\bar{X}_j - \bar{X}_k}{\sqrt{MSW / \bar{n}}}$$

where n = average sample size per group

- **Decision rule: Q is compared with a theoretical Tukey' s Studentized critical value to decide a statistical significance.**

Tukey's studentized method

- Studentized range statistic

$$Q_{k,n-k,\alpha} = \frac{\max \bar{X}_i - \min \bar{X}_i}{\sqrt{WMS}} \sqrt{N}$$

- The difference between X_1 and X_2 is declared if

$$Q_{ij} = \frac{|\bar{X}_i - \bar{X}_j| \sqrt{N}}{\sqrt{WMS}} > Q_{k,n-k,\alpha}$$

- When the sample sizes are not the same,

$$N = 2n_i n_j / (n_i + n_j)$$

Linear contrasts

In some cases, we are interested in *weighted* difference between groups, and linear contrasts offer a better way to make formal comparisons:

$$C_1 = X_1 - X_2$$

$$C_2 = X_2 - X_4$$

$$C_3 = (X_1 + X_2)/2 - X_4$$

$$C_4 = (X_1 + X_2)/2 - (X_3 + X_4)/2$$

$$C_5 = (n_1 X_1 + n_2 X_2)/(n_1 + n_2) - (n_3 X_3 + n_4 X_4)/(n_3 + n_4)$$

In general: $C_i = \sum c_i \bar{X}_i$ where the sum of $c_i = 0$

Linear contrasts

In general: $C_i = \sum c_i \bar{X}_i$ where the sum of $c_i = 0$

| | c_1 | c_2 | c_3 | c_4 |
|--|-------|-------|-------|-------|
| $C_1 = X_1 - X_2$ | 1 | -1 | 0 | 0 |
| $C_2 = X_2 - X_4$ | 0 | 1 | 0 | -1 |
| $C_3 = (X_1 + X_2)/2 - X_4$ | 0.5 | 0.5 | 0 | -1 |
| $C_4 = (X_1 + X_2)/2 - (X_3 + X_4)/2$ | 0.5 | 0.5 | -0.5 | -0.5 |
| $C_5 = (n_1 X_1 + n_2 X_2)/(n_1 + n_2) - (n_3 X_3 + n_4 X_4)/(n_3 + n_4)$ | | | | |
| $c_1 = n_1/(n_1 + n_2); c_2 = n_2/(n_1 + n_2); c_3 = -n_3/(n_3 + n_4); c_4 = -n_4/(n_3 + n_4)$ | | | | |

The standard error of C_i is:

$$SE(C_i) = \sqrt{MSW \times \sum \frac{c_i^2}{n_i}}$$

Scheffé' s method

$$L = C / SE(C)$$

C is judged to be significantly different from 0 if $L > S$, where

$$S = \sqrt{(k - 1) F_{k-1, N-k, \alpha}}$$

Bonferroni's method

$$L = C / SE(C)$$

C is judged to be significantly different from 0 if $L > B$, where

$$B = t_{1-\alpha/2m, n-k}$$

Where m is the number of planned comparisons

Dunnett's test for comparison with a control group

- There are situations where we might want to compare our experimental groups to one control group.
- This results in fewer comparisons.
- The Dunnett's test is designed for this purpose

Dunnett's test for comparison with a control group

- Steps in calculation:

- Arrange the group means in descending order

$$L_i = \frac{\bar{X}_i - \bar{X}_0}{\sqrt{WMS}} \sqrt{\frac{n_0 n_i}{n_0 + n_i}}$$

- Calculate contrast L_i

- Where n_0 and n_i are sample size of the control and comparing group, respectively.
- If $L_i >$ theoretical L , the difference is declared “significant”

Which one is appropriate?

- **LSD: control for per-comparison error rate**
- **Tukey's HSD: control for type I per-experiment error rate**
- **Bonferroni, Duncan's t-test: control for type I per-experiment error rate, but it has a higher type II error rate than Tukey's procedure.**
- **Scheffe's test: control for type I per-experiment error rate, but it has a higher type II error rate than Tukey's procedure.**

Which one is appropriate?

- If all pairwise comparisons are of interest, the Tukey's procedure is preferred to Bonferroni's;
- If not all pairwise comparisons are to be considered, the Bonferroni's procedure may be better.
- The Bonferroni's procedure is better than Scheffé's when the number of contrasts to be estimated is about the same as the number of groups or less.
- In any given problem, the method with the narrowest confidence interval is preferred.

Multiple Comparisons: Tukey's Method

```
res <- aov(y ~ group)
```

```
TukeyHSD (res)
```

```
Tukey multiple comparisons of means
```

```
95% family-wise confidence level
```

```
Fit: aov(formula = y ~ group)
```

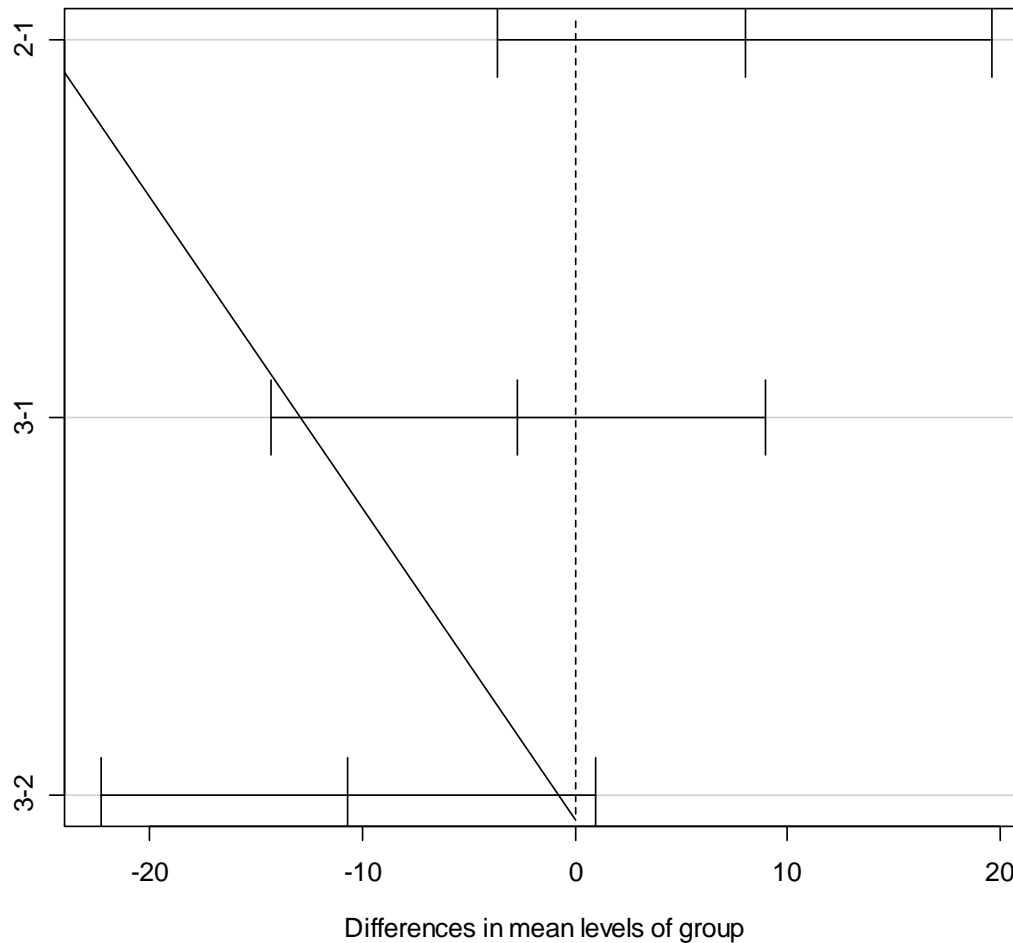
```
$group
```

| | diff | lwr | upr | p adj |
|-----|------------|------------|------------|-----------|
| 2-1 | 8.000000 | -3.661237 | 19.6612370 | 0.1689400 |
| 3-1 | -2.666667 | -14.327904 | 8.9945703 | 0.7714179 |
| 3-2 | -10.666667 | -22.327904 | 0.9945703 | 0.0692401 |

Multiple Comparisons: Tukey's Method

```
plot(TukeyHSD(res), ordered=T)
```

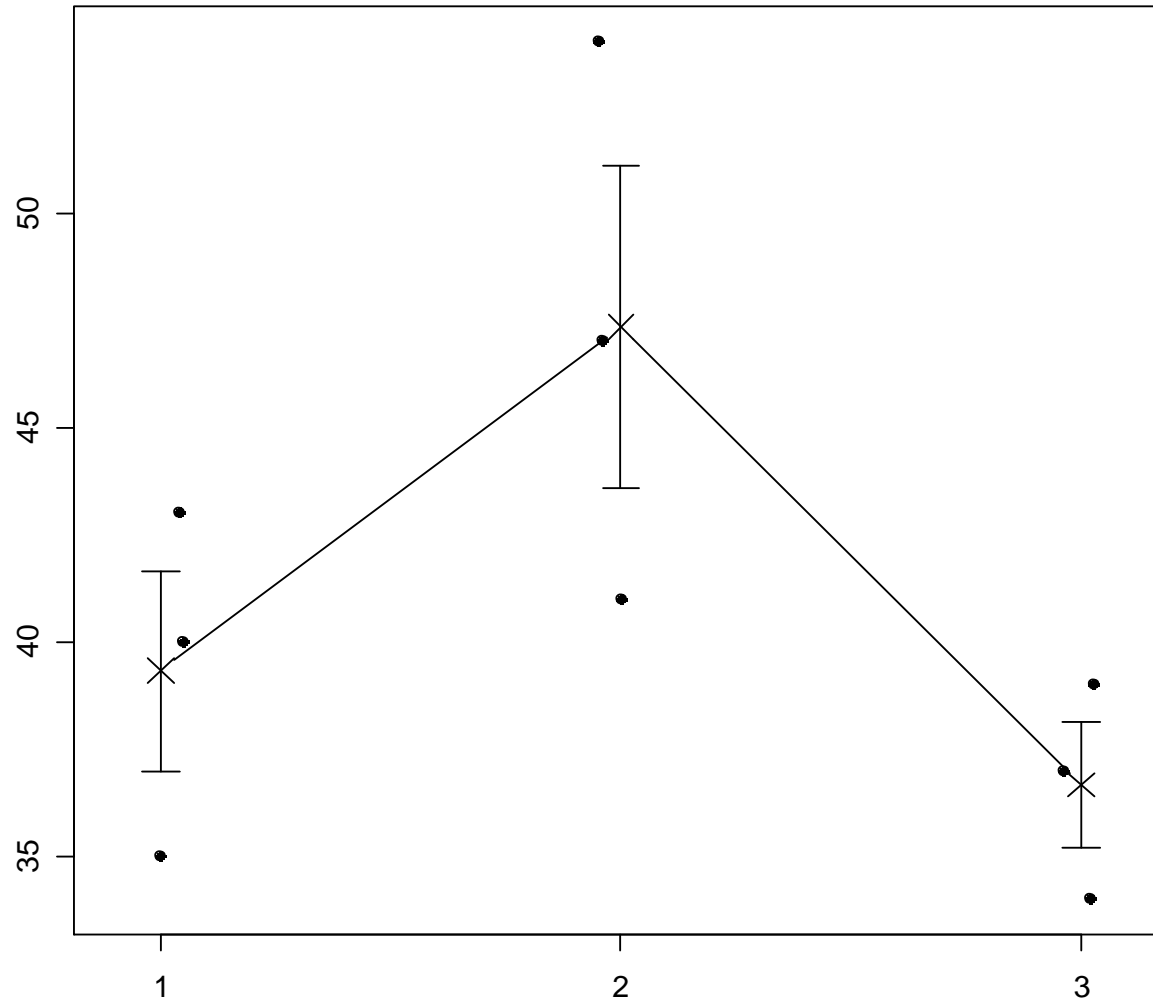
95% family-wise confidence level



Graphical Analysis

```
average <- tapply(y, group, mean)
std <- tapply(y, group, sd)
ss <- tapply(y, group, length)
sem <- std/sqrt(ss)
stripchart(y ~ group, "jitter", jit=0.05, pch=16,
  vert=TRUE)
arrows(1:3, average+sem, 1:3, average-sem, angle=90,
  code=3, length=0.1)
lines(1:3, average, pch=4, type="b", cex=2)
```

Graphical Analysis



Summary

- **Unpaired t-test is suitable for comparing means of two independent groups.**
- **One-way ANOVA is used for comparing means of more than 2 groups.**
- **Analyses must be planned**
 - **Detailed enough to answer the question of interest**
- **Planned analyses must be carried out and reported**
 - **Scientific integrity, honesty**
- **Avoid “data snooping”**

More ANOVA

Factorial ANOVA

| Variety | Pesticide | | | | Total |
|---------|-----------|-----|-----|-----|-------|
| | 1 | 2 | 3 | 4 | |
| B1 | 29 | 50 | 43 | 53 | 175 |
| B2 | 41 | 58 | 42 | 73 | 214 |
| B3 | 66 | 85 | 63 | 85 | 305 |
| Tổng số | 136 | 193 | 154 | 211 | 694 |

Model:

$$\text{product} = a + b(\text{variety}) + g(\text{pesticide}) + e$$

Factorial ANOVA by R

| Variety | Pesticide | | | | Total |
|---------|-----------|-----|-----|-----|-------|
| | 1 | 2 | 3 | 4 | |
| B1 | 29 | 50 | 43 | 53 | 175 |
| B2 | 41 | 58 | 42 | 73 | 214 |
| B3 | 66 | 85 | 63 | 85 | 305 |
| Tổng số | 136 | 193 | 154 | 211 | 694 |

```
variety <- c(1, 1, 1, 1, 2, 2, 2, 2, 3, 3, 3, 3)
pesticide <- c(1, 2, 3, 4, 1, 2, 3, 4, 1, 2, 3, 4)
product <- c(29, 50, 43, 53, 41, 58, 42, 73, 66, 85, 69, 85)
```

```
variety <- as.factor(variety)
pesticide <- as.factor(pesticide)
data <- data.frame(variety, pesticide, product)
```

Factorial ANOVA by R

```
analysis <- aov(product ~ variety + pesticide)
anova (analysis)
```

Analysis of Variance Table

Response: product

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|-----------|----|---------|---------|---------|----------|-----|
| variety | 2 | 2225.17 | 1112.58 | 44.063 | 0.000259 | *** |
| pesticide | 3 | 1191.00 | 397.00 | 15.723 | 0.003008 | ** |
| Residuals | 6 | 151.50 | 25.25 | | | |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons

```
> TukeyHSD (analysis)
```

```
Tukey multiple comparisons of means
```

```
95% family-wise confidence level
```

```
Fit: aov(formula = product ~ variety + pesticide)
```

```
$variety
```

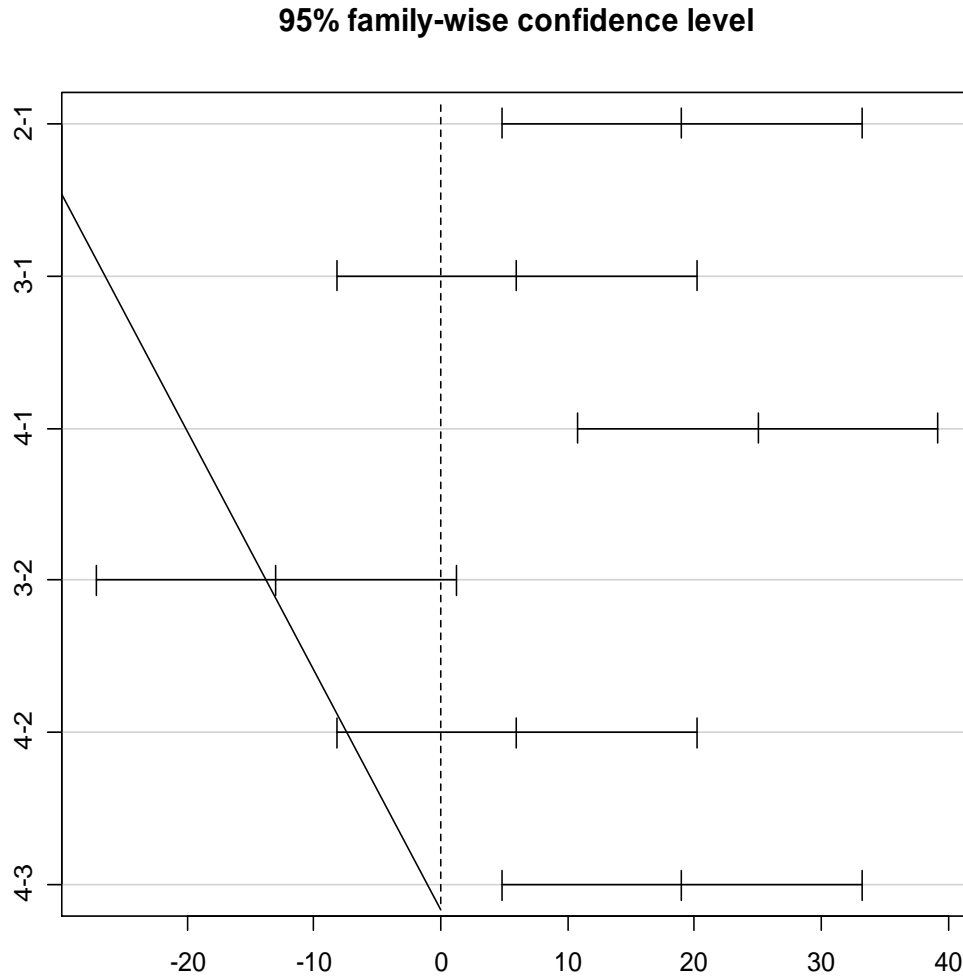
| | diff | lwr | upr | p adj |
|-----|-------|-----------|----------|-----------|
| 2-1 | 9.75 | -1.152093 | 20.65209 | 0.0749103 |
| 3-1 | 32.50 | 21.597907 | 43.40209 | 0.0002363 |
| 3-2 | 22.75 | 11.847907 | 33.65209 | 0.0016627 |

```
$pesticide
```

| | diff | lwr | upr | p adj |
|-----|------|------------|-----------|-----------|
| 2-1 | 19 | 4.797136 | 33.202864 | 0.0140509 |
| 3-1 | 6 | -8.202864 | 20.202864 | 0.5106152 |
| 4-1 | 25 | 10.797136 | 39.202864 | 0.0036109 |
| 3-2 | -13 | -27.202864 | 1.202864 | 0.0704233 |
| 4-2 | 6 | -8.202864 | 20.202864 | 0.5106152 |
| 4-3 | 19 | 4.797136 | 33.202864 | 0.0140509 |

Multiple Comparisons

```
> plot(TukeyHSD (analysis) , ordered=TRUE)
```



Latin-square ANOVA

| Plot | Variety | | | |
|------|-----------|-----------|-----------|-----------|
| | 1 | 2 | 3 | 4 |
| 1 | 175 Aa | 143 Ba | 128 Bb | 166 Ab |
| 2 | 170 Ab | 178 Aa | 140 Ba | 131 Bb |
| 3 | 135 Bb | 173 Ab | 169 Aa | 141 Ba |
| 4 | 145 Ba | 136 Bb | 165 Ab | 173 Aa |

Latin-square ANOVA: summary

| Plot | Variety | | | |
|------|-----------|-----------|-----------|-----------|
| | 1 | 2 | 3 | 4 |
| 1 | 175 Aa | 143 Ba | 128 Bb | 166 Ab |
| 2 | 170 Ab | 178 Aa | 140 Ba | 131 Bb |
| 3 | 135 Bb | 173 Ab | 169 Aa | 141 Ba |
| 4 | 145 Ba | 136 Bb | 165 Ab | 173 Aa |

| Mean by variety | Mean by plot | Mean by method |
|---|---|---|
| 1: 156.25 2: 157.50 3: 150.50 4: 152.75 Overall mean: 154.25 | 1: 153.00 2: 154.75 3: 154.50 4: 154.75 Overall mean: 154.25 | 1 (Aa): 173.75 2 (Ab): 168.50 3 (Ba): 142.25 4 (Bb): 132.50 Overall mean: 154.25 |

Latin-square ANOVA by R

| Plot | Variety | | | |
|------|-----------|-----------|-----------|-----------|
| | 1 | 2 | 3 | 4 |
| 1 | 175 Aa | 143 Ba | 128 Bb | 166 Ab |
| 2 | 170 Ab | 178 Aa | 140 Ba | 131 Bb |
| 3 | 135 Bb | 173 Ab | 169 Aa | 141 Ba |
| 4 | 145 Ba | 136 Bb | 165 Ab | 173 Aa |

```
y <- c(175, 143, 128, 166, 170, 178, 140, 131, 135, 173, 169, 141, 145, 136, 165, 173)
```

```
variety <- c(1,2,3,4, 1,2,3,4, 1,2,3,4, 1,2,3,4,)
```

```
sample <- c(1,1,1,1, 2,2,2,2, 3,3,3,3, 4,4,4,4)
```

```
method <- c(1, 3, 4, 2, 2, 1, 3, 4, 4, 2, 1, 3, 3, 4, 2, 1)
```

```
variety <- as.factor(variety)
```

```
sample <- as.factor(sample)
```

```
method <- as.factor(method)
```

Latin-square ANOVA by R

```
latin <- aov(y ~ sample + variety + method)
summary(latin)
```

| Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|-----------|--------|---------|-----------|-----------|-----|
| sample | 8.5 | 2.8 | 2.2667 | 0.1810039 | |
| variety | 123.5 | 41.2 | 32.9333 | 0.0004016 | *** |
| method | 4801.5 | 1600.5 | 1280.4000 | 8.293e-09 | *** |
| Residuals | 7.5 | 1.3 | | | |

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.'
                 0.1 ' ' 1
```

Latin-square – Multiple Comparisons

```
> TukeyHSD(latin)
```

```
$variety
```

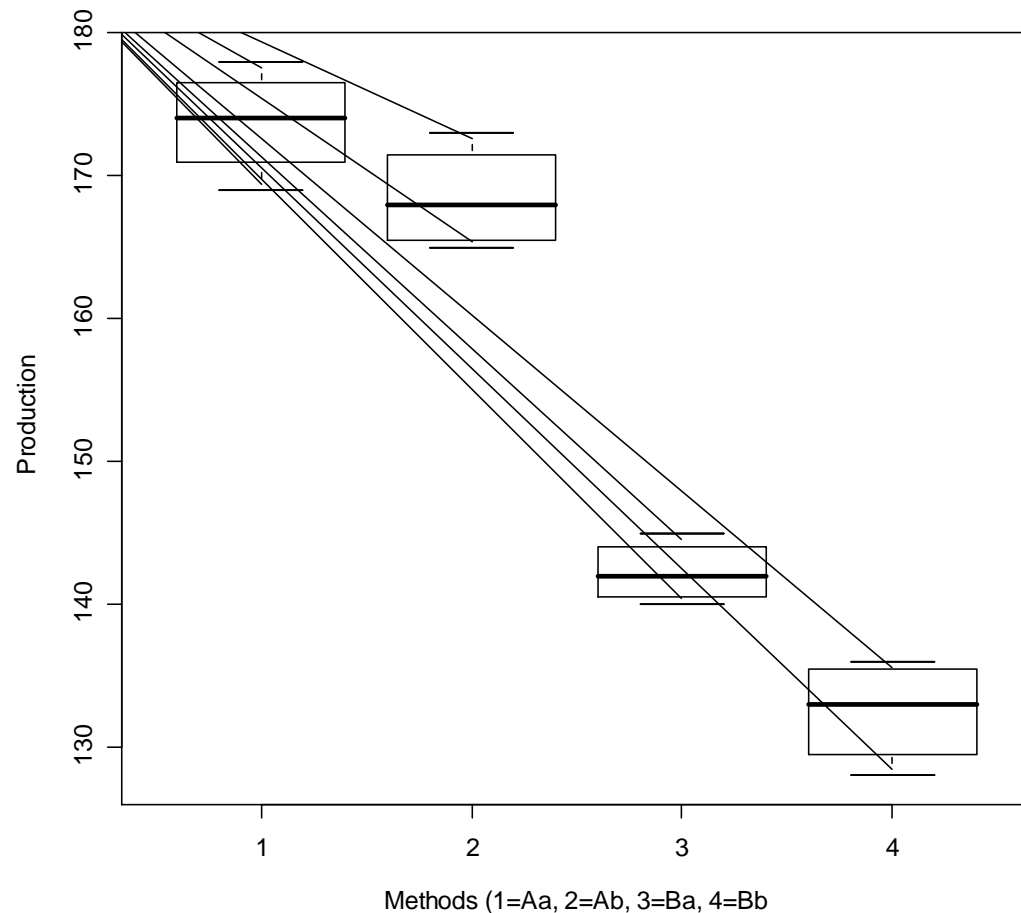
| | diff | lwr | upr | p adj |
|-----|-------|------------|------------|-----------|
| 2-1 | 1.25 | -1.4867231 | 3.9867231 | 0.4528549 |
| 3-1 | -5.75 | -8.4867231 | -3.0132769 | 0.0014152 |
| 4-1 | -3.50 | -6.2367231 | -0.7632769 | 0.0173206 |
| 3-2 | -7.00 | -9.7367231 | -4.2632769 | 0.0004803 |
| 4-2 | -4.75 | -7.4867231 | -2.0132769 | 0.0038827 |
| 4-3 | 2.25 | -0.4867231 | 4.9867231 | 0.1034761 |

```
$method
```

| | diff | lwr | upr | p adj |
|-----|--------|------------|------------|-----------|
| 2-1 | -5.25 | -7.986723 | -2.513277 | 0.0023016 |
| 3-1 | -31.50 | -34.236723 | -28.763277 | 0.0000001 |
| 4-1 | -41.25 | -43.986723 | -38.513277 | 0.0000000 |
| 3-2 | -26.25 | -28.986723 | -23.513277 | 0.0000004 |
| 4-2 | -36.00 | -38.736723 | -33.263277 | 0.0000000 |
| 4-3 | -9.75 | -12.486723 | -7.013277 | 0.0000730 |

Graphical Analysis

```
boxplot(y ~ method, xlab="Methods (1=Aa, 2=Ab, 3=Ba, 4=Bb)",  
        ylab="Production")
```



Cross-over Study ANOVA

| Nhóm | Mã số bệnh nhân số (id) | Thời gian (phút) ra mồ hôi trên trán | |
|-----------|-------------------------|--------------------------------------|----------------|
| | | Tháng 1 | Tháng 2 |
| AB | | A | Placebo |
| | 1 | 6 | 4 |
| | 3 | 8 | 7 |
| | 5 | 12 | 6 |
| | 6 | 7 | 8 |
| | 9 | 9 | 10 |
| | 10 | 6 | 4 |
| | 13 | 11 | 6 |
| | 15 | 8 | 8 |
| BA | | Placebo | A |
| | 2 | 5 | 7 |
| | 4 | 9 | 6 |
| | 7 | 7 | 11 |
| | 8 | 4 | 7 |
| | 11 | 9 | 8 |
| | 12 | 5 | 4 |
| | 14 | 8 | 9 |
| | 16 | 9 | 13 |

Cross-over Study ANOVA by R

```
y <- c(6,8,12,7,9,6,11,8, 4,7,6,8,10,4,6,8, 5,9,7,4,9,5,8,9
      7,6,11,7,8,4,9,13)
seq <- c(1,1,1,1,1,1,1,1, 1,1,1,1,1,1,1, 2,2,2,2,2,2,2,2,
      2,2,2,2,2,2,2,2)
period <- c(1,1,1,1,1,1,1,1, 2,2,2,2,2,2,2, 2,2,2,2,2,2,2,2,
      1,1,1,1,1,1,1,1)
treat <- c(1,1,1,1,1,1,1,1, 2,2,2,2,2,2,2, 1,1,1,1,1,1,1,1,
      2,2,2,2,2,2,2,2)
id <- c(1,3,5,6,9,10,13,15, 1,3,5,6,9,10,13,15, 2,4,7,8,11,12,14,16,
      2,4,7,8,11,12,14,16)

seq <- as.factor(seq)
period <- as.factor(period)
treat <- as.factor(treat)
id <- as.factor(id)

data <- data.frame(seq, period, treat, id, y)
```

Cross-over Study ANOVA by R

```
xover <- lm(y ~ treat + seq + period)
anova(xover)
```

Analysis of Variance Table

Response: y

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|-----------|----|---------|---------|---------|---------|---|
| treat | 1 | 16.531 | 16.531 | 4.9046 | 0.04388 | * |
| seq | 1 | 0.031 | 0.031 | 0.0093 | 0.92466 | |
| period | 1 | 0.781 | 0.781 | 0.2318 | 0.63764 | |
| id | 14 | 103.438 | 7.388 | 2.1921 | 0.07711 | . |
| Residuals | 14 | 47.187 | 3.371 | | | |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Cross-over Study ANOVA by R

```
> TukeyHSD(aov(y ~ treat+seq+period+id))
```

```
Tukey multiple comparisons of means
```

```
95% family-wise confidence level
```

```
Fit: aov(formula = y ~ treat + seq + period + id)
```

```
$treat
```

| | diff | lwr | upr | p adj |
|-----|---------|-----------|-------------|-----------|
| 2-1 | -1.4375 | -2.829658 | -0.04534186 | 0.0438783 |

```
$seq
```

| | diff | lwr | upr | p adj |
|-----|--------|-----------|----------|----------|
| 2-1 | 0.0625 | -1.329658 | 1.454658 | 0.924656 |

```
$period
```

| | diff | lwr | upr | p adj |
|-----|---------|-----------|----------|-----------|
| 2-1 | -0.3125 | -1.704658 | 1.079658 | 0.6376395 |