Two Way Analysis of Variance

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- If an experiment has a quantitative outcome and two categorical explanatory variables A and B that are defined in such a way that each experimental unit (subject) can be exposed to any combination of one level of one explanatory variable and one level of the other explanatory variable, then the most common analysis method is two-way ANOVA.
- Because there are two different explanatory variables the effects on the outcome of a change in one variable may either not depend on the level of the other variable (additive model) or it may depend on the level of the other variable (interaction model).

- Goal: compare the means of a single variable at different levels of two factors A and B in scientific experiments .
- Suppose factor A has a levels and factor B has b levels
- In total we have ab treatments
- We assume that each treatment level, we have n_{ij} experimental units and let Y_{ijk} be the kth observation when $A = i$ and $B = j$
- We will assume first that $n_{ij} \equiv n$ for all (i,j) (we say that the design is balanced)
- We assume that the Y_{ijk} are independent and that

$$Y_{ijk} \sim N(\mu_{ij}, \sigma^2)$$

Let

$$\mu_{i\bullet} = \frac{\sum_{j=1}^{b} \mu_{ij}}{b}, \quad \mu_{\bullet j} = \frac{\sum_{i=1}^{a} \mu_{ij}}{a}, \quad \mu_{\bullet\bullet} = \frac{\sum_{i=1}^{a}\sum_{j=1}^{b} \mu_{ij}}{ab}$$

we can represent the means as follows

		B				
		B_1	B_2	$\dots$	B_b	
	A_1	μ_{11}	μ_{12}	$\dots$	μ_{1b}	$\mu_{1\bullet}$
	A_2	μ_{21}	μ_{22}	$\dots$	μ_{2b}	$\mu_{2\bullet}$
A	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
	A_a	μ_{a1}	μ_{a2}	$\dots$	μ_{ab}	$\mu_{2\bullet}$
		$\mu_{\bullet 1}$	$\mu_{\bullet 2}$	$\dots$	$\mu_{\bullet b}$	$\mu_{\bullet\bullet}$

- Let

$$\bar{Y}_{ij\bullet} = \frac{\sum_{k=1}^{n} Y_{ijk}}{n} \quad \bar{Y}_{\bullet j\bullet} = \frac{\sum_{i=1}^{a}\sum_{k=1}^{n} Y_{ijk}}{an}$$

$$\bar{Y}_{i\bullet\bullet} = \frac{\sum_{j=1}^{b}\sum_{k=1}^{n} Y_{ijk}}{kn} \quad \bar{Y}_{\bullet\bullet\bullet} = \frac{\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} Y_{ijk}}{abn}$$

- Let also

$$SST = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(Y_{ijk} - \bar{Y}_{\bullet\bullet\bullet})^2 \quad SSA = nb\sum_{i=1}^{k}(\bar{Y}_{i\bullet\bullet} - \bar{Y}_{\bullet\bullet\bullet})^2$$

$$SSB = na\sum_{i=1}^{k}(\bar{Y}_{i\bullet\bullet} - \bar{Y}_{\bullet\bullet\bullet})^2 \quad SSAB = n\sum_{i=1}^{k}(\bar{Y}_{ij\bullet} - \bar{Y}_{i\bullet\bullet} - \bar{Y}_{\bullet j\bullet} + \bar{Y}_{\bullet\bullet\bullet})^2$$

$$SSE = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(Y_{ijk} - \bar{Y}_{ij\bullet})^2$$

Then

$$SST = SSA + SSB + SSAB + SSE$$

ANOVA table

Source	SS	df	MS	E(MS)
Factor A	SSA	a-1	$\frac{SSA}{a-1}$	$\sigma^2 + \frac{bn\sum_{i=1}^{a}(\mu_{i\bullet}-\mu_{\bullet\bullet})^2}{a-1}$
Factor B	SSB	b-1	$\frac{SSB}{b-1}$	$\sigma^2 + \frac{an\sum_{i=1}^{a}(\mu_{\bullet j}-\mu_{\bullet\bullet})^2}{b-1}$
Factor AB	SSAB	(a-1)(b-1)	$\frac{SSAB}{(a-1)(b-1)}$	$\sigma^2 + \frac{n\sum_{i=1}^{a}\sum_{j=1}^{b}(\mu_{ij}-\mu_{i\bullet}-\mu_{\bullet j}+\mu_{\bullet\bullet})^2}{(a-1)(b-1)}$
Error	SSE	ab(n-1)	$\frac{SSE}{ab(n-1)}$	σ^2
Total	SST	abn-1		

F-test

- Notice that

$$\frac{E(MSA)}{E(MSE)} = 1 \quad \Leftrightarrow \quad \sum_{i=1}^{a} (\mu_{i\bullet} - \mu_{\bullet\bullet})^2 = 0$$
$$\Leftrightarrow \quad \mu_{1\bullet} = \mu_{2\bullet} = \ldots = \mu_{a\bullet}$$

- We should always start with testing the interaction. If interaction is present, then we ignore main effects look at it as one factor with ab levels
- if no interaction, compare levels of A ignoring B and compare levels of B ignoring A

F-test

- Test for interaction

$$H_0 : \text{No interation and} H_a : \text{Yes interaction}$$

- The test statistic is

$$F = \frac{MSAB}{MSE}$$

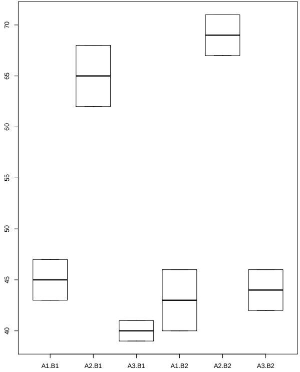
- Reject H_0 if

$$F > F(1-\alpha, (a-1)(b-1), ab(n-1))$$

or if p-value less than α

```
> response<-c(47,43, 62,68, 41,39, 46,40,67,71,42,46)
> A<-c(rep(c(rep("A1",2),rep("A2",2),rep("A3",2)) ,2 ))
> B<-c(rep("B1",6),rep("B2",6))
>boxplot(response~A*B)
```

Figure: Box Plots

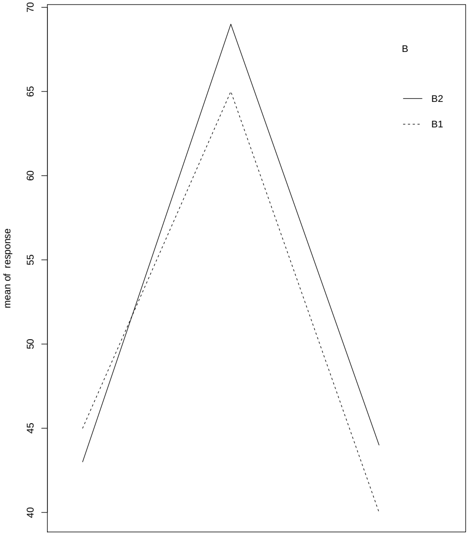


Example

```
> tapply(response, list(A),mean)
A1 A2 A3
44 67 42
> tapply(response, list(B),mean)
B1 B2
50 52
> interaction.plot(A,B,response)
   B1 B2
A1 45 43
A2 65 69
A3 40 44
```

Example

Figure: Interaction Plot



```
> summary(aov(response~A*B))
            Df Sum Sq Mean Sq F value   Pr(>F)
A            2   1544   772.0  74.710 5.75e-05
B            1     12    12.0   1.161    0.323
A:B          2     24    12.0   1.161    0.375
Residuals    6     62    10.3

A           ***
B
A:B
```

We have $SSA =, 1544 SSB = 12, SSAB = 24, SSE = 62$. their degrees of freedom are, 2, 1, 2 and 6, respectively

The p-value of the test for interaction is 0.375. We fail to reject H_0 and conclude that there is no interaction.

```
Model without interaction:
> summary(aov(response~A+B))
            Df Sum Sq Mean Sq F value   Pr(>F)
A            2   1544   772.0  71.814 7.75e-06
B            1     12    12.0   1.116    0.322
Residuals    8     86    10.8
```

From this output we can conclude that A level means (p-value¡0.05) are different but B level means (p-value=0.322) are not (There an A effect but no B effect) We drop B and refit the model

Example

```
> summary(aov(response~A))
            Df Sum Sq Mean Sq F value  Pr(>F)
A            2   1544   772.0    70.9 3.1e-06
Residuals    9     98    10.9
```

Factor A is significant at $\alpha = 0.01$

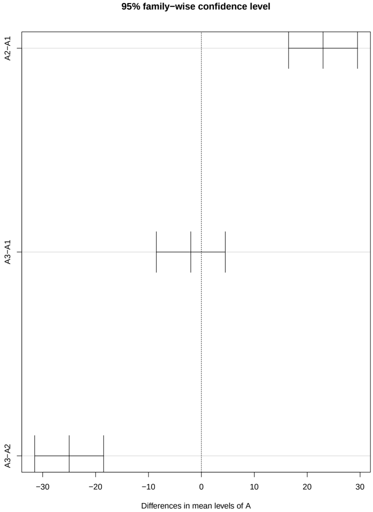
```
> fit<-aov(response~A)
> tk<-TukeyHSD(fit, "A")
> plot(tk)
> tk
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = response ~ A)

$A
      diff       lwr       upr     p adj
A2-A1   23  16.48532  29.51468 0.0000108
A3-A1   -2  -8.51468   4.51468 0.6789461
A3-A2  -25 -31.51468 -18.48532 0.0000054
```

So we have two groups here $\{A_1, A_3\}$ and $\{A_2\}$.

Figure: Tukey method based confidence intervals



```
data example;
input   response A $   B $ ;
datalines;
47     A1    B1
43     A1    B1
62     A1    B2
68     A1    B2
41     A2    B1
39     A2    B1
46     A2    B2
40     A2    B2
67     A3    B1
71     A3    B1
42     A3    B2
46     A3    B2
;
proc glm;
class A B;
model response= A B A*B;
run;
```

- We have

$$\begin{aligned} Y_{ijk} &= \mu_{ij} + \epsilon_{ijk} \\ &= \mu_{\bullet\bullet} + \mu_{i\bullet} - \mu_{\bullet\bullet} + \mu_{\bullet j} - \mu_{\bullet\bullet} + \mu_{ij} - \mu_{i\bullet} - \mu_{\bullet j} + \mu_{\bullet\bullet} + \epsilon_{ijk} \\ &= \mu_{\bullet\bullet} + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk} \end{aligned}$$

  where

$$\alpha_i = \mu_{i\bullet} - \mu_{\bullet\bullet}, \quad \beta_j = \mu_{\bullet j} - \mu_{\bullet\bullet} \quad \text{and} \quad (\alpha\beta)_{ij} = \mu_{ij} - \mu_{i\bullet} - \mu_{\bullet j} + \mu_{\bullet\bullet}$$

  and

$$\sum_{i=1}^{a} \alpha_i = 0, \quad \sum_{j=1}^{b} \beta_j = 0, \quad \text{and} \sum_{i=1}^{a} (\alpha\beta)_{ij} = \sum_{j=1}^{b} (\alpha\beta)_{ij} = 0$$

- The αs measure the effect of factor A, the βs measure the effect of factor B and the $(\alpha\beta)_{ij}$s are the interaction terms

Suppose $a = b = 2$.

$$\mu_{11} = \mu_{\bullet\bullet} + \alpha_1 + \beta_1 + (\alpha\beta)_{11} \qquad \mu_{12} = \mu_{\bullet\bullet} + \alpha_1 + \beta_2 + (\alpha\beta)_{12}$$
$$\mu_{21} = \mu_{\bullet\bullet} + \alpha_2 + \beta_1 + (\alpha\beta)_{21} \qquad \mu_{22} = \mu_{\bullet\bullet} + \alpha_2 + \beta_2 + (\alpha\beta)_{22}$$

Note that since
$\alpha_1+\alpha_2 = 0, \beta_1+\beta_2 = 0, (\alpha\beta)_{11}+(\alpha\beta)_{12} = 0, (\alpha\beta)_{21}+(\alpha\beta)_{22} = 0, (\alpha\beta)_{11}+(\alpha\beta)_{21} = 0$ and $(\alpha\beta)_{12} + (\alpha\beta)_{22} = 0$ we have

Factor Effects Model

$$\begin{array}{ll} \mu_{11} = \mu_{\bullet\bullet} + \alpha_1 + \beta_1 + (\alpha\beta)_{11} & \mu_{12} = \mu_{\bullet\bullet} + \alpha_1 - \beta_1 - (\alpha\beta)_{11} \\ \mu_{21} = \mu_{\bullet\bullet} - \alpha_1 + \beta_1 - (\alpha\beta)_{11} & \mu_{22} = \mu_{\bullet\bullet} - \alpha_1 - \beta_1 + (\alpha\beta)_{11} \end{array}$$

from the model we have

$$\begin{aligned} \mu_{11} - \mu_{21} &= (\mu_{\bullet\bullet} + \alpha_1 + \beta_1 + (\alpha\beta)_{11}) - (\mu_{\bullet\bullet} - \alpha_1 + \beta_1 - (\alpha\beta)_{11}) \\ &= 2\alpha_1 + 2(\alpha\beta)_{11} \end{aligned}$$

and

$$\begin{aligned} \mu_{12} - \mu_{22} &= (\mu_{\bullet\bullet} + \alpha_1 - \beta_1 - (\alpha\beta)_{11}) - (\mu_{\bullet\bullet} - \alpha_1 - \beta_1 + (\alpha\beta)_{11}) \\ &= 2\alpha_1 - 2(\alpha\beta)_{11} \end{aligned}$$

So when

$$2\alpha_1 + 2(\alpha\beta)_{11} = 2\alpha_1 - 2(\alpha\beta)_{11}$$

we have

$$\begin{aligned} \mu_{11} - \mu_{21} = \mu_{12} - \mu_{22} \quad &\Leftrightarrow \quad 2\alpha_1 + 2(\alpha\beta)_{11} = 2\alpha_1 - 2(\alpha\beta)_{11} \\ &\Leftrightarrow \quad (\alpha\beta)_{11} = 0 \end{aligned}$$

If you fix the level of one factor and look at the means, difference does not depend on that level if there is no inteaction

Factor Effects Model

In general

$$\mu_{ij} - \mu_{i'j} = \mu_{ij'} - \mu_{i'j'} \quad \text{for all } i, i', j, j' \quad \Leftrightarrow \quad (\alpha\beta)_{ij} = 0 \quad \text{for all} \quad (i, j)$$

	A1	A2	B	A1B	A2B
A1 B1	1	0	1	1	0
A1 B1	1	0	1	1	0
A2 B1	0	1	1	0	1
A2 B1	0	1	1	0	1
A3 B1	-1	-1	1	-1	-1
A3 B1	-1	-1	1	-1	-1
A1 B2	1	0	-1	-1	0
A1 B2	1	0	-1	-1	0
A2 B2	0	1	-1	0	-1
A2 B2	0	1	-1	0	-1
A3 B2	-1	-1	-1	1	1
A3 B2	-1	-1	-1	1	1

Use regression techniques and test

$$H_0 : \text{reduced model versus} \qquad H_a : \text{full model}$$

use partial F-test

```
Fit Full Model
> fit1<-lm(response~A1+A2+B+A1*B+A2*B)
> summary(fit1)

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   51.000      0.928  54.959 2.44e-09 ***
A1            -7.000      1.312  -5.334  0.00177 **
A2            16.000      1.312  12.192 1.85e-05 ***
B             -1.000      0.928  -1.078  0.32261
A1:B           2.000      1.312   1.524  0.17835
A2:B          -1.000      1.312  -0.762  0.47494
---
Residual standard error: 3.215 on 6 degrees of freedom
Multiple R-squared:  0.9622,Adjusted R-squared:  0.9308
F-statistic: 30.58 on 5 and 6 DF,  p-value: 0.0003384
```

```
Fit Reduced Model
> fit2<-lm(response~A1+A2+B)
> summary(fit2)

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  51.0000     0.9465  53.884 1.56e-11 ***
A1           -7.0000     1.3385  -5.230 0.000793 ***
A2           16.0000     1.3385  11.953 2.21e-06 ***
B            -1.0000     0.9465  -1.057 0.321579

Residual standard error: 3.279 on 8 degrees of freedom
Multiple R-squared:  0.9476,Adjusted R-squared:  0.928
F-statistic: 48.25 on 3 and 8 DF,  p-value: 1.813e-05
```

```
> anova(fit2,fit1)
Analysis of Variance Table

Model 1: response ~ A1 + A2 + B
Model 2: response ~ A1 + A2 + B + A1 * B + A2 * B
  Res.Df RSS Df Sum of Sq      F Pr(>F)
1      8  86
2      6  62  2        24 1.1613 0.3747
```

Example

```
bloodpresure<-c(158, 163, 173,178,168,188,183,198,178,193,186,191,
196,181,176,185,190,195,200,180)

biofeedback<-factor(c(rep("present",10),rep("absent",10)))

drug<-factor(rep (c(rep("present",5),rep("absent",5)),2))

bpdata<-data.frame(bloodpresure, biofeedback,drug)
```

```
print(bpdata)
  bloodpresure biofeedback    drug
1          158     present present
2          163     present present
3          173     present present
4          178     present present
5          168     present present
6          188     present  absent
7          183     present  absent
8          198     present  absent
9          178     present  absent
10         193     present  absent
11         186      absent present
12         191      absent present
13         196      absent present
14         181      absent present
15         176      absent present
16         185      absent  absent
17         190      absent  absent
18         195      absent  absent
19         200      absent  absent
20         180      absent  absent
```

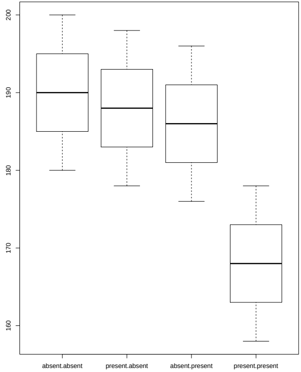
Example

```
> summary(bpdata)
 bloodpresure      biofeedback       drug
Min.   :158.0   absent :10    absent :10
1st Qu.:177.5   present:10    present:10
Median :184.0
Mean   :183.0
3rd Qu.:191.5
Max.   :200.0
```

Example

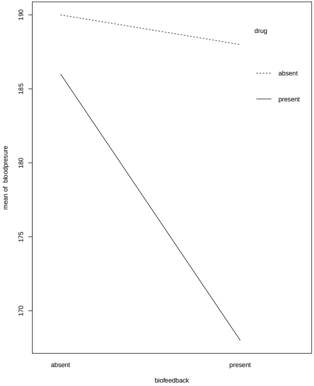
```
> interaction.plot(biofeedback, drug, bloodpresure)
```

Figure: Box Plots



```
> interaction.plot(biofeedback, drug, bloodpresure)
```

Figure: Interation Plots



Example

```
> myanova<-aov(bloodpresure~biofeedback*drug)

>summary(myanova)

                Df Sum Sq Mean Sq F value  Pr(>F)
biofeedback      1    500   500.0    8.00 0.01211 *
drug             1    720   720.0   11.52 0.00371 **
biofeedback:drug 1    320   320.0    5.12 0.03792 *
Residuals       16   1000    62.5
---
Signif. codes:  0 ?***? 0.001 ?**? 0.01 ?*? 0.05 ?.? 0.1 ? ? 1
```

Example

```
> TukeyHSD(myanova, which="biofeedback:drug")
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = bloodpresure ~ biofeedback * drug)

$`biofeedback:drug`
                               diff      lwr       upr     p adj
present:absent-absent:absent     -2 -16.3051 12.305099 0.9775889
absent:present-absent:absent     -4 -18.3051 10.305099 0.8534038
present:present-absent:absent   -22 -36.3051 -7.694901 0.0022719
absent:present-present:absent    -2 -16.3051 12.305099 0.9775889
present:present-present:absent  -20 -34.3051 -5.694901 0.0051230
present:present-absent:present  -18 -32.3051 -3.694901 0.0115535
```

When the design is unbalanced, we need to use Type III sum of squares (to be explained in class) as follows

```
install.packages("car")
library(car)
model<-lm(bloodpresure~biofeedback*drug,
contrasts=list(biofeedback= contr.sum, drug=contr.sum))
Anova(model, type=3)
```

Example

```
 y<-c(10,12,11,3,4,5,6,7,8,8,7)
 a<-c(rep("a1",6),rep("a2",5))
 b<-c(rep("b1",4),rep("b2",2), rep("b1",2),rep("b2",3))

 aov(y~a+b)
Call:
   aov(formula = y ~ a + b)

Terms:
                       a         b            Residuals
Sum of Squares   0.24545   8.35263           71.94737
Deg. of Freedom        1         1                  8

Residual standard error: 2.998903
Estimated effects may be unbalanced
```

```
> aov(y~b+a)
Call:
   aov(formula = y ~ b + a)

Terms:
                       b         a   Residuals
Sum of Squares   8.51212   0.08596   71.94737
Deg. of Freedom        1         1          8

Residual standard error: 2.998903
Estimated effects may be unbalanced
```

Notice that the sums of squares depends on the oder in which a and b are entered into the model

```
library(car)
model<-lm(x~a+b, contrasts=list(a= contr.sum, b=contr.sum))
Anova(model, type=3)
Anova Table (Type III tests)

Response: x
            Sum Sq Df F value    Pr(>F)
(Intercept) 576.91  1 64.1480 4.331e-05 ***
a             0.09  1  0.0096    0.9245
b             8.35  1  0.9287    0.3634
Residuals    71.95  8
---
Signif. codes:  0 ?***? 0.001 ?**? 0.01 ?*? 0.05 ?.? 0.1 ? ? 1
```