

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 k th 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	...	B_b	
A	A_1	μ_{11}	μ_{12}	...	μ_{1b}	$\mu_{1\bullet}$
	A_2	μ_{21}	μ_{22}	...	μ_{2b}	$\mu_{2\bullet}$
	\vdots	\vdots	\vdots	\vdots	\vdots	\vdots
	\vdots	\vdots	\vdots	\vdots	\vdots	\vdots
	\vdots	\vdots	\vdots	\vdots	\vdots	\vdots
	A_a	μ_{a1}	μ_{a2}	...	μ_{ab}	$\mu_{a\bullet}$
		$\mu_{\bullet 1}$	$\mu_{\bullet 2}$...	$\mu_{\bullet b}$	$\mu_{\bullet\bullet}$

- Let

$$\begin{aligned}\bar{Y}_{ij\bullet} &= \frac{\sum_{k=1}^n Y_{ijk}}{n} & \bar{Y}_{\bullet j\bullet} &= \frac{\sum_{i=1}^a \sum_{k=1}^n Y_{ijk}}{an} \\ \bar{Y}_{i\bullet\bullet} &= \frac{\sum_{j=1}^b \sum_{k=1}^n Y_{ijk}}{kn} & \bar{Y}_{\bullet\bullet\bullet} &= \frac{\sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^n Y_{ijk}}{abn}\end{aligned}$$

- Let also

$$\begin{aligned}SST &= \sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^n (Y_{ijk} - \bar{Y}_{\bullet\bullet\bullet})^2 & SSA &= nb \sum_{i=1}^a (\bar{Y}_{i\bullet\bullet} - \bar{Y}_{\bullet\bullet\bullet})^2 \\ SSB &= na \sum_{j=1}^b (\bar{Y}_{\bullet j\bullet} - \bar{Y}_{\bullet\bullet\bullet})^2 & SSAB &= n \sum_{i=1}^a \sum_{j=1}^b (\bar{Y}_{ij\bullet} - \bar{Y}_{i\bullet\bullet} - \bar{Y}_{\bullet j\bullet} + \bar{Y}_{\bullet\bullet\bullet})^2 \\ SSE &= \sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^n (Y_{ijk} - \bar{Y}_{ij\bullet})^2\end{aligned}$$

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	$\frac{SSB}{b-1}$	$\sigma^2 + \frac{an \sum_{j=1}^b (\mu_{\bullet j} - \mu_{\bullet\bullet})^2}{b-1}$
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		

- Notice that

$$\begin{aligned} \frac{E(MSA)}{E(MSE)} = 1 &\Leftrightarrow \sum_{i=1}^a (\mu_{i\bullet} - \mu_{\bullet\bullet})^2 = 0 \\ &\Leftrightarrow \mu_{1\bullet} = \mu_{2\bullet} = \dots = \mu_{a\bullet} \end{aligned}$$

- 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

- Test for interaction

H_0 : No interaction and H_a : Yes interaction

- The test statistic is

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

- Reject H_0 if

$$F > F_{\gamma}((a-1)(b-1), ab(n-1))$$

or if p-value less than γ

- here we assume there no interaction effect in which case the degrees of freedom of SSE is $abn - a - b + 1$

$$H_0 : \text{No A effect} \quad H_a : \text{there is a A effect}$$

- The test statistic is

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

- Reject H_0 if

$$F > F_{\gamma}(a - 1, abn - a - b + 1)$$

or if p-value less than γ

- here we also assume there no interaction effect in which case the degrees of freedom of SSE is $abn - a - b + 1$

$$H_0 : \text{No B effect} \quad H_a : \text{there is a B effect}$$

- The test statistic is

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

- Reject H_0 if

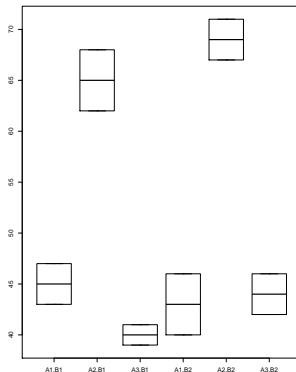
$$F > F_{\gamma}(b - 1, abn - a - b + 1)$$

or if p-value less than γ

Example

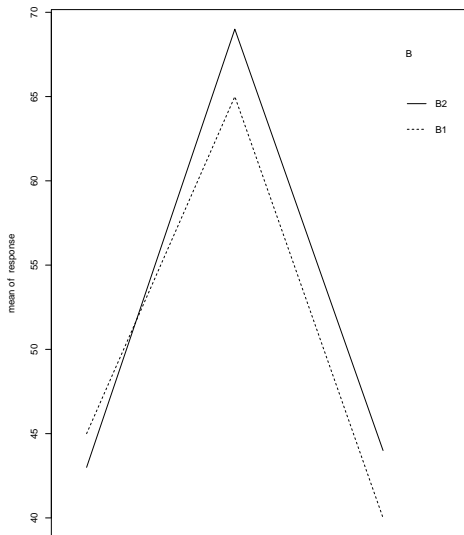
```
> response<-c(47,43, 62,68, 41,39, 46,40,67,71,42,46)
> A<-c(rep(c(rep("A1",2),rep("A2",2),rep("A3",2)),2 ))
> B<-c(rep("B1",6),rep("B2",6))
> boxplot(response~A*B)
```

Figure: Box Plots



```
> 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
```

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 ($p\text{-value} < 0.05$) are different but B level means ($p\text{-value} = 0.322$) are not (There an A effect but no B effect) We drop B and refit the model


```
> 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 $\gamma = 0.01$

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

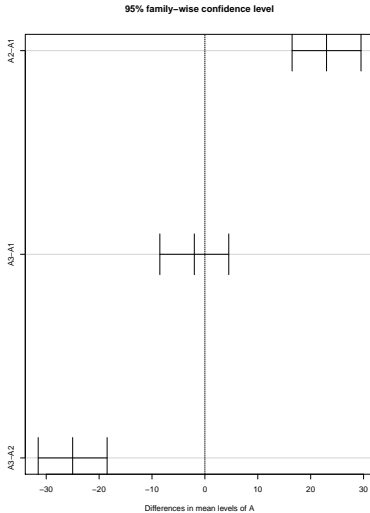
```
Fit: aov(formula = response ~ A)
```

```
$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

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

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

Suppose $a = b = 2$.

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

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

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

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

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

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

In general

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

			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

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

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

```
bloodpressure<-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(bloodpressure, biofeedback,drug)
```

Example

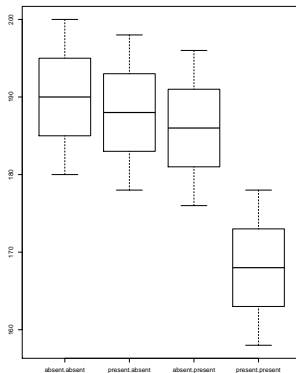
```
print(bpdata)
  bloodpressure biofeedback    drug
1          158      present present
2          163      present present
3          173      present present
4          178      present present
5          168      present present
6          188      present  absent
7          183      present  absent
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)
  bloodpressure    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
```

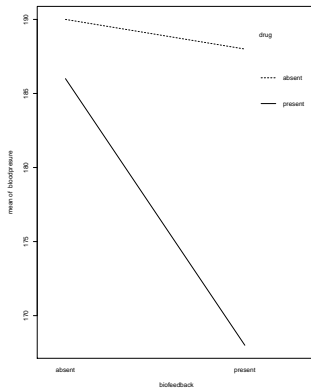
```
> interaction.plot(biofeedback, drug, bloodpressure)
```

Figure: Box Plots



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

Figure: Iteration Plots




```
> myanova<-aov(bloodpressure~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
```

```
> TukeyHSD(myanova, which="biofeedback:drug")
```

```
Tukey multiple comparisons of means
```

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

```
Fit: aov(formula = bloodpressure ~ 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(bloodpressure~biofeedback*drug,  
contrasts=list(biofeedback= contr.sum, drug=contr.sum))  
Anova(model, type=3)
```

```
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 order 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

