

Two Way Analysis of Variance

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- 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

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

- 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

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

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

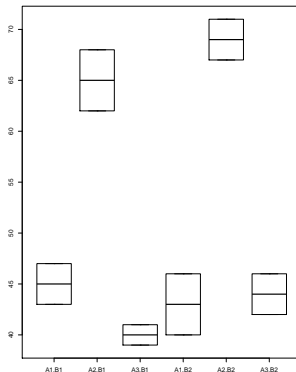
$$F > F(1 - \alpha, (a - 1)(b - 1), ab(n - 1))$$

or if p-value less than α

Example

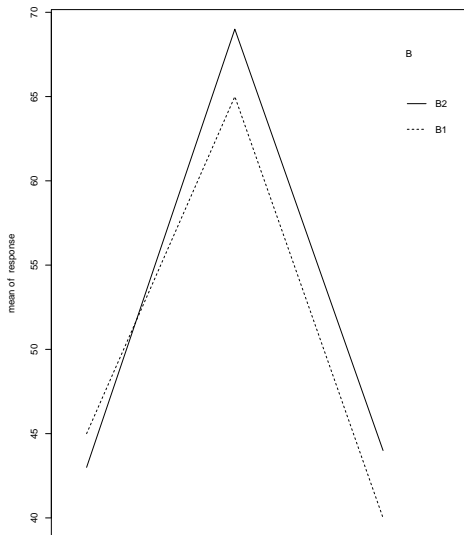
```
> response<-c(47,43, 62,68, 41,39, 46,40,67,71,42,46)
> A<-c(rep(c(rep("A1",2),rep("A2",2),rep("A3",2)),2 ))
> B<-c(rep("B1",6),rep("B2",6))
> boxplot(response~A*B)
```

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 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 ($p\text{-value} \leq 0.05$) 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 $\alpha = 0.01$

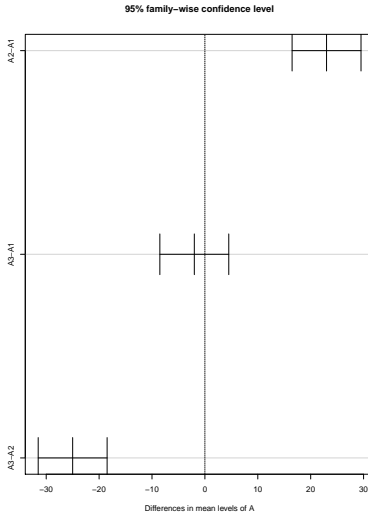
```
> fit<-aov(response~A)
> tk<-TukeyHSD(fit, "A")
> plot(tk)
> tk
  Tukey multiple comparisons of means
    95% family-wise confidence level
```

```
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

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