

Experimental Design and Statistical Methods



Workshop

FACTORIAL DESIGNS and NESTED DESIGNS

Jesús Piedrafita Arilla

jesus.piedrafita@uab.cat

Departament de Ciència Animal i dels Aliments

UAB

Items

- Factorial design
 - Concept and interpretation of interaction
- Nested design
- Basic commands
 - aov – anova
 - summary lm
 - fixed
 - random
 - interaction.plot

Factorial designs

When we are interested in contrasting the effect of **two or more main factors**, and the possible joint effect –the **interaction effect**–, we use factorial designs. An example of the simplest 2×2 factorial design is the following:

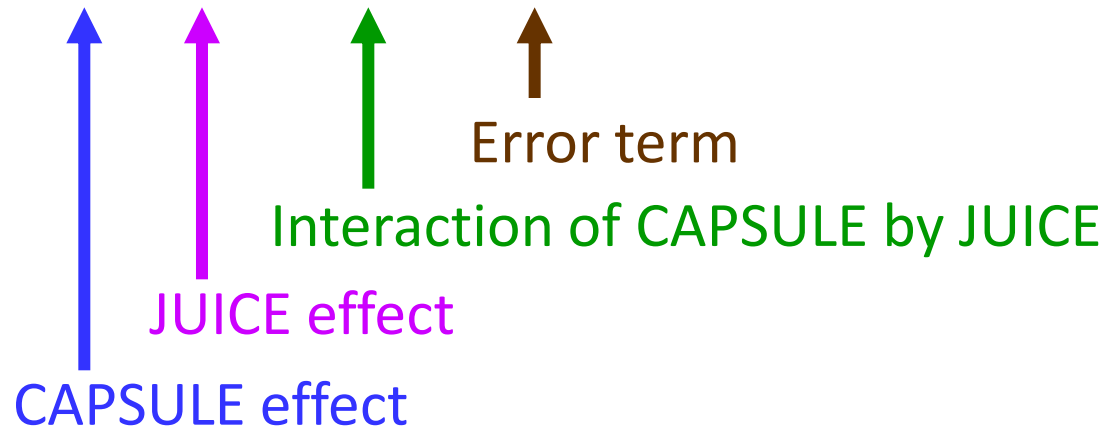
	Type C	Type V
Gastric	39.5	47.4
	45.7	43.5
	49.8	39.8
	50.2	36.1
	63.8	41.2
Duodenal	31.2	44.0
	33.5	41.2
	36.7	47.3
	42.0	45.3
	38.1	42.7

Data come from an experiment to test the solubility of two types of capsules (C and V) depending upon the juice of the gastrointestinal tract (Gastric or Duodenal). The variable measured as indicator of solubility is the time to observe the first bubbles.

Factorial designs – model

The model for this design is described as follows:

$$y_{ijk} = \mu + \tau_i + \varphi_j + \tau\varphi_{ij} + \varepsilon_{ijk}$$



Capsule and juice are said in general **main effects**.

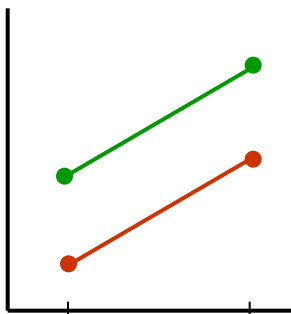
The model can include three or more main effects and their interactions (of two factors, three factors and higher levels).

Interaction

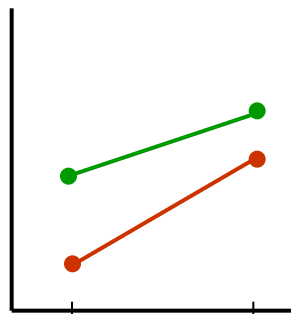
The dependence of the effect of one factor on the levels of another factor is called **interaction**.

The sum of squares for interaction measures the **departure of the subgroup means from the values expected on the basis of additive combinations of the row and column means**.

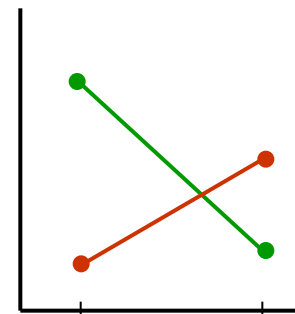
Any given combination of levels of factors may result in a positive or negative deviation from the expected value based on the means of the levels of the factors. If this deviation is positive we talk of **synergism**; if negative, **interference**. Both tend to magnify the interaction SS.



No interaction



Quantitative interaction



Qualitative interaction

Factorial designs – summary statistics and boxplots

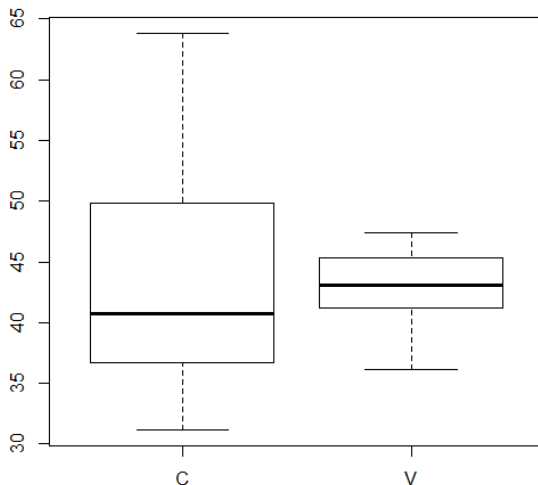
```
> summary(SOLUB.CAP)
```

JUICE	CAPSULE	SOLUB
DUO:10	C:10	Min. :31.20
GAS:10	V:10	1st Qu.:39.15
		Median :42.35
		Mean :42.95
		3rd Qu.:46.10
		Max. :63.80

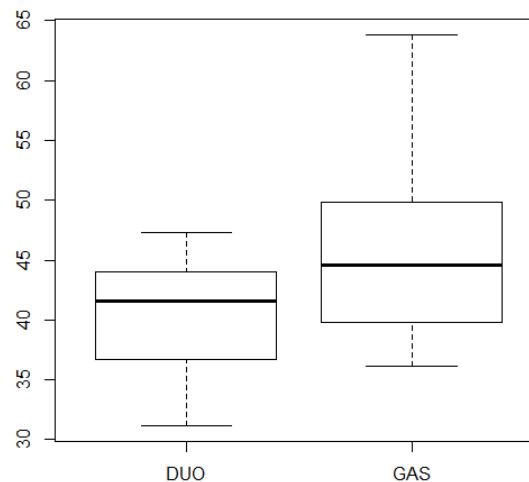
According to the boxplots, there is no evidence of non normality and also no apparent relationship between mean and variance.

Observe an outlier in GASxC.

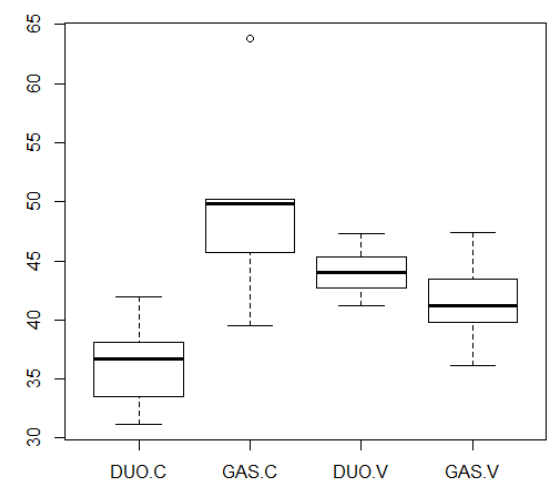
```
boxplot(SOLUB~CAPSULE)
```



```
boxplot(SOLUB~JUICE)
```



```
boxplot(SOLUB~JUICE*CAPSULE)
```



Factorial designs – results (1) -

```
> SOLUB.AOV<-aov(SOLUB~CAPSULE*JUICE)
> anova(SOLUB.AOV)
```

Note that **CAPSULE*JUICE** is equivalent to **CAPSULE + JUICE + CAPSULE:JUICE**

Analysis of Variance Table

Response: SOLUB

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
CAPSULE	1	0.20	0.20	0.0066	0.936055
JUICE	1	151.25	151.25	5.0232	0.039542 *
CAPSULE:JUICE	1	320.00	320.00	10.6277	0.004916 **
Residuals	16	481.76	30.11		

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

We see that JUICE is significant but CAPSULE is not. Indeed the interaction is significant.

Contrast of levels of the significant factor, in this case JUICE must be done within each level of the other factor.

When interaction is not significant, it can be removed from the model and run a new model with the two main effects only.

Factorial designs – results (2) -

```
> summary.lm(SOLUB.AOV)
```

```
...
```

```
Coefficients:
```

	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	36.300	2.454	14.792	9.42e-11	***
CAPSULEV	7.800	3.470	2.248	0.03906	*
JUICEGAS	13.500	3.470	3.890	0.00130	**
CAPSULEV:JUICEGAS	-16.000	4.908	-3.260	0.00492	**

```
---
```

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

```
Residual standard error: 5.487 on 16 degrees of freedom
```

```
Multiple R-squared: 0.4946,    Adjusted R-squared: 0.3998
```

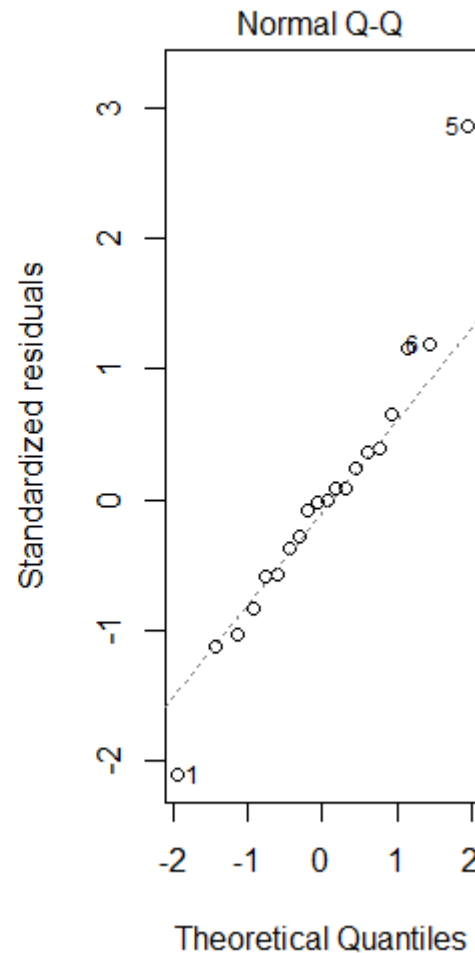
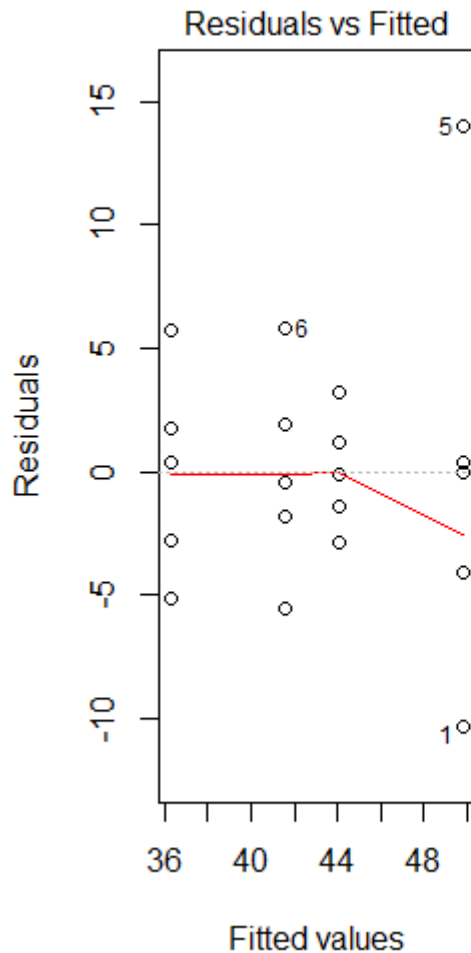
```
F-statistic: 5.219 on 3 and 16 DF,  p-value: 0.01054
```

The model explains 49.46% of the total variability ($R^2 = 0.4946$)

Factorial designs – diagnostics -

```
>layout(matrix(c(1,2),1,2))  
>plot(SOLUB.AOV, which=c(1,2))
```

which= selects this kind of graphic for an anova object



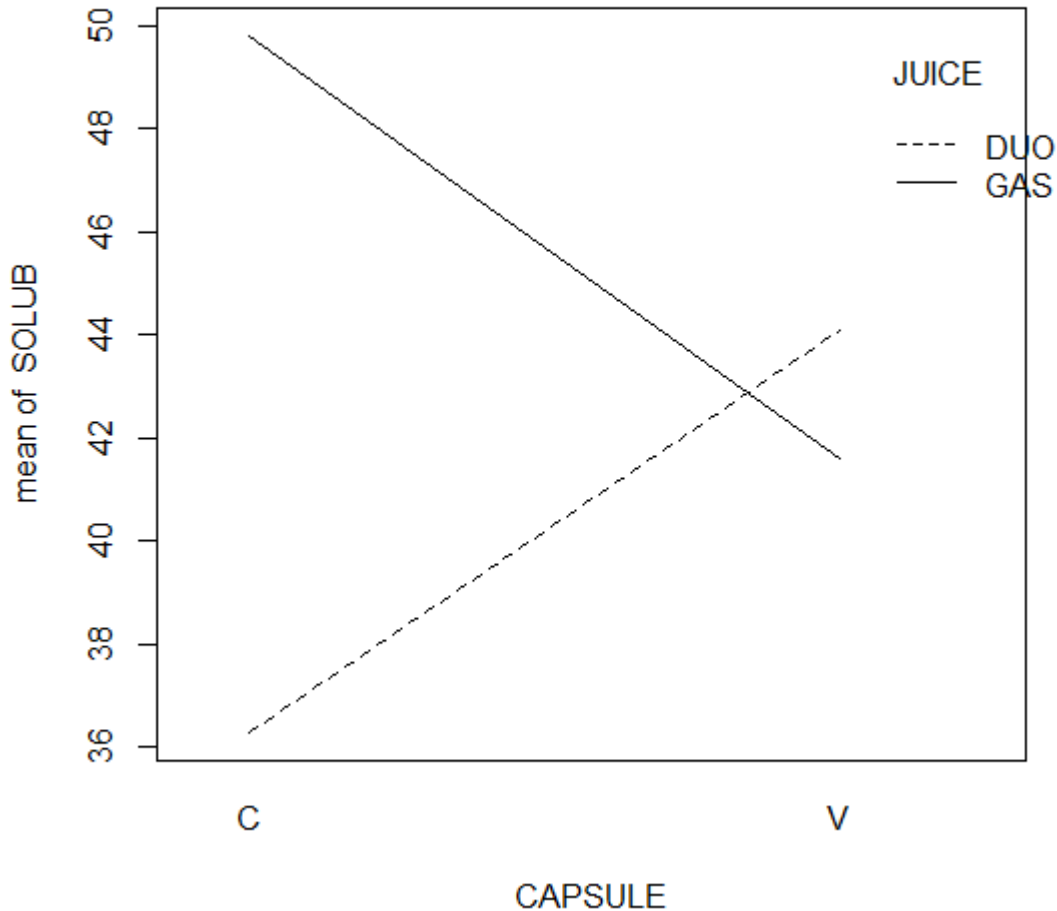
This graphic evaluates the overall residual and predicted values for the interaction effect.

There is a random distribution (independence) of residuals among the fitted values.

The distribution of residuals does not deviate much of normality.

Factorial designs – graphic representation of interaction -

```
> interaction.plot(CAPSULE, JUICE, SOLUB)
```



```
> tapply(SOLUB,  
+ list(CAPSULE, JUICE), mean)
```

	DUO	GAS
C	36.3	49.8
V	44.1	41.6

This is a clear example of **qualitative interaction** between the two factors.

Comparison of means

```
> TukeyHSD(aov(SOLUB ~ CAPSULE*JUICE))
```

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

```
$CAPSULE
```

	diff	lwr	upr	p adj
V-C	-0.2	-5.402197	5.002197	0.9360548

```
$JUICE
```

	diff	lwr	upr	p adj
GAS-DUO	5.5	0.2978026	10.7022	0.0395423

```
$`CAPSULE:JUICE`
```

	diff	lwr	upr	p adj
V:DUO-C:DUO	7.8	-2.129017	17.729017	0.1526843
C:GAS-C:DUO	13.5	3.570983	23.429017	0.0064094
V:GAS-C:DUO	5.3	-4.629017	15.229017	0.4452697
C:GAS-V:DUO	5.7	-4.229017	15.629017	0.3843072
V:GAS-V:DUO	-2.5	-12.429017	7.429017	0.8875433
V:GAS-C:GAS	-8.2	-18.129017	1.729017	0.1251515

Remember that due to the presence of interaction, comparisons between levels of main effects (CAPSULE and JUICE) have no sense.

We need to make comparisons between combinations of the levels.

Nested design

Imagine that we have put our cows in two separate and independent farms, chosen at random, for each treatment. This is a **NESTED** or hierarchical design, that can be summarized as follows:

T1		T2		T3	
F1	F2	F3	F4	F5	F6
3.7	3.6	4.5	3.9	4.5	4.4
3.9	3.4	4.3	4.1	4.7	4.1
4.5	4.1	4.7	3.7	4.4	3.9
4.3	4.3	4.8	4.1	4.6	4.0
4.1	4.2	4.3	4.0	4.3	4.4

A less convenient way to present these data is as in our right. This array shows how an analysis similar to the previous one would have given many missing cells.

	T1	T2	T3
Farm 1	3.7		
	3.9		
	4.5		
	4.3		
	4.1		
Farm 2	3.6		
	3.4		
	4.1		
	4.3		
	4.2		
Farm 3		4.5	
		4.3	
		4.7	
		4.8	
		4.3	
Farm 4		3.9	
		4.1	
		3.7	
		4.1	
		4.0	
Farm 5			4.5
			4.7
			4.4
			4.6
			4.3
Farm 6			4.4
			4.1
			3.9
			4.0
			4.4

Nested designs – model -

The model for this design is described as follows:

$$y_{ijk} = \mu + \tau_i + \varphi(\tau)_{ij} + \varepsilon_{ijk}$$

TRT effect

FARM (within TRT effect)

Error term

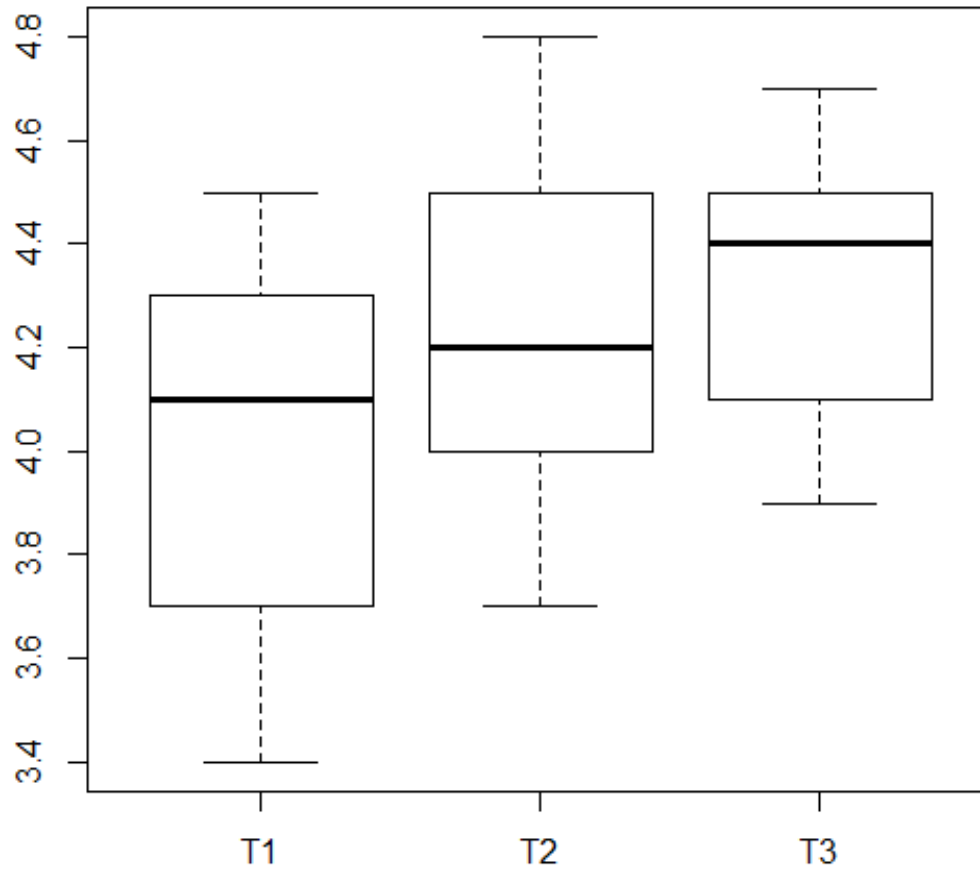
In some cases, we can be interested in contrasting the farm effects included in the model and then **farm is defined as a fixed effect**.

In another cases, however, we are not interested in the particular effect of each farm and then **define farm as a random effect** (nuisance factor).

In genetic applications, such as the estimation of heritability, both effects are defined as random (not analysed here).

Nested designs – Boxplots -

```
> boxplot(FAT~TRT)
```



No obvious deviations from normality are observed.

Nested designs – results with farm fixed -

```
> summary(FATN.F<-aov(FAT~TRT/FARM))
```

Farm is defined
as **fixed** effect

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	2	0.5447	0.2723	3.937	0.03320 *
TRT:FARM	3	1.1540	0.3847	5.561	0.00482 **
Residuals	24	1.6600	0.0692		

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

Significant

Observe that both TRT and TRT:FARM Mean Sq are contrasted against the Residuals Mean Sq.

Treatment effect, as well as farm effect, are significant.

Nested designs – results with farm random -

```
> summary(FATN.R<-aov(FAT ~ TRT + Error(FARM)))
```

Farm is defined as **random effect**

Error: FARM

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	2	0.5447	0.2723	0.708	0.56
Residuals	3	1.1540	0.3847		

The treatment effects (not significant) are weaker than the between farm variability (significant)

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Residuals	24	1.66	0.06917	5.56	0.0041

Observe that TRT is contrasted with FARM as the error term and that FARM is contrasted with the residual error term.

$$\frac{0.3847}{0.06917}$$

```
> df(5.56166, 3, 24)
[1] 0.004054074
```

Not in the R output. It can be obtained also from the previous analysis.