Experimental Design and Statistical Methods
R *Workshop*

Factorial designs and nested designs

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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:

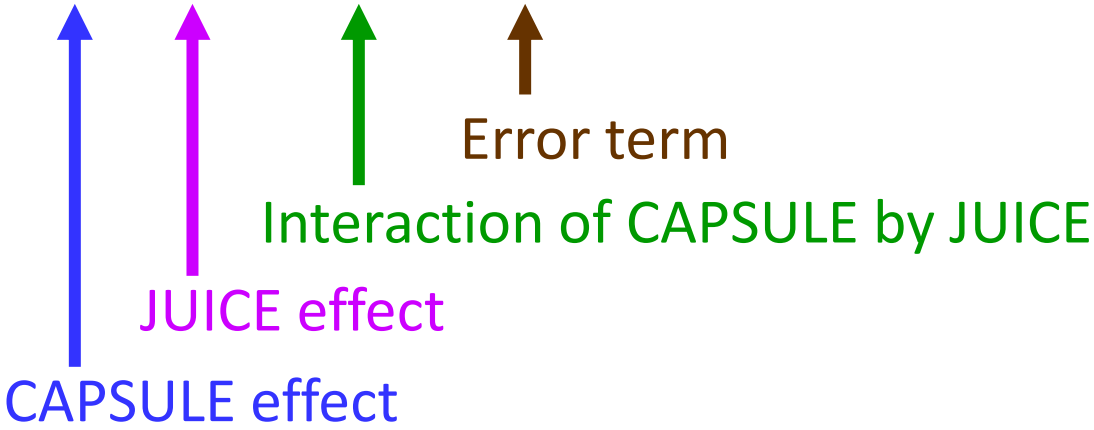
	Type C	**Type V**
Gastric	39.5	47.4
	45.7	43.5
	49.8	39.8
	50.2	36.1
	63.8	41.2
Duodenal	31.2	44.0
	33.5	41.2
	36.7	47.3
	42.0	45.3
	38.1	42.7

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:

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



Error term

Interaction of CAPSULE by JUICE

JUICE effect

CAPSULE effect

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

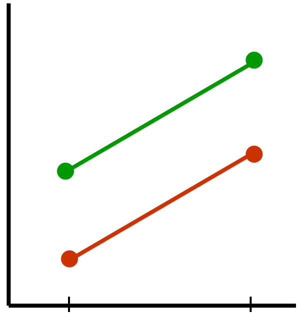
The model can include three or more main effects and their interactions (of two factors, three factors and higher levels).

Interaction

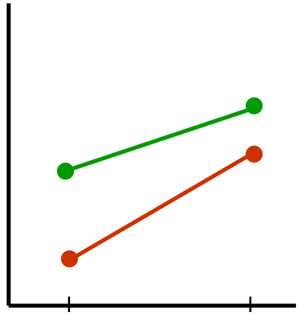
The dependence of the effect of one factor on the levels of another factor is called **interaction**.

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**.

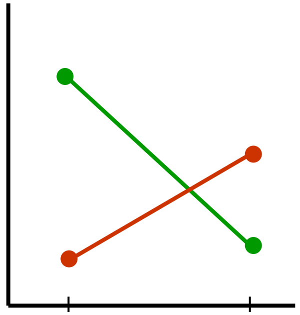
Any given combination of levels of factors may result in a positive or negative deviation from the expected value based on the means of the levels of the factors. If this deviation is positive we talk of **synergism**; if negative, **interference**. Both tend to magnify the interaction SS.



No interaction



Quantitative interaction



Qualitative interaction

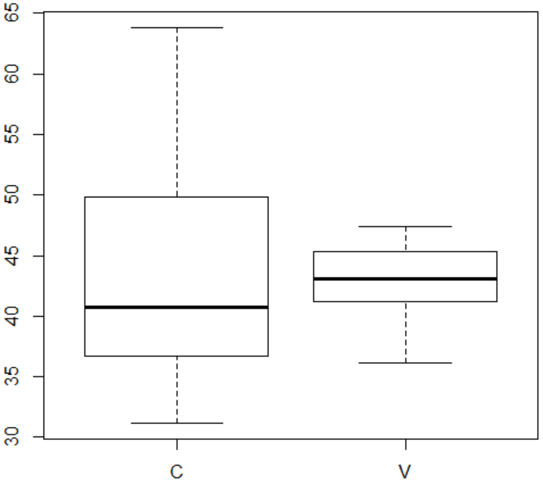
Factorial designs – summary statistics and boxplots

```
> 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
```

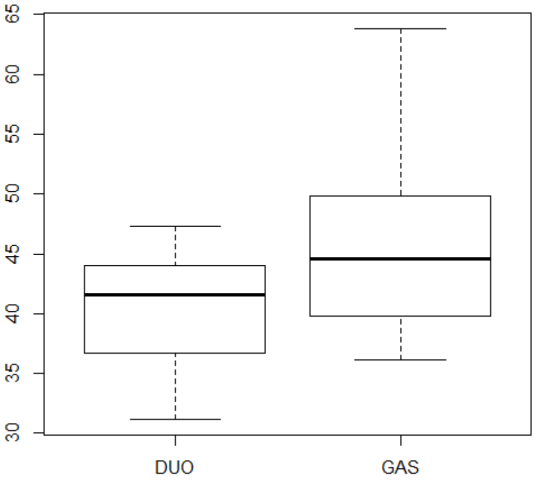
According to the boxplots, there is no evidence of non normality and also no apparent relationship between mean and variance.

Observe an outlier in GASxC.

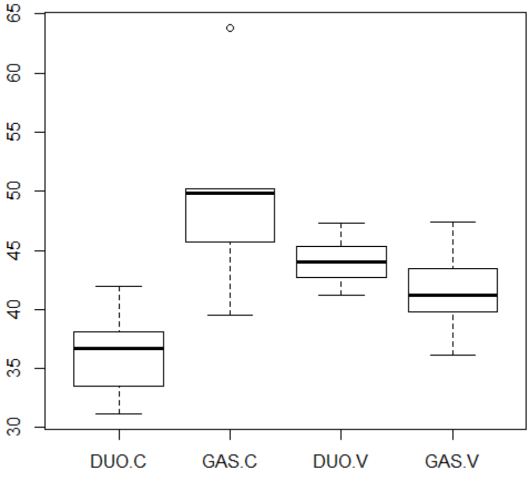
`boxplot(SOLUB~CAPSULE)`



`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) -

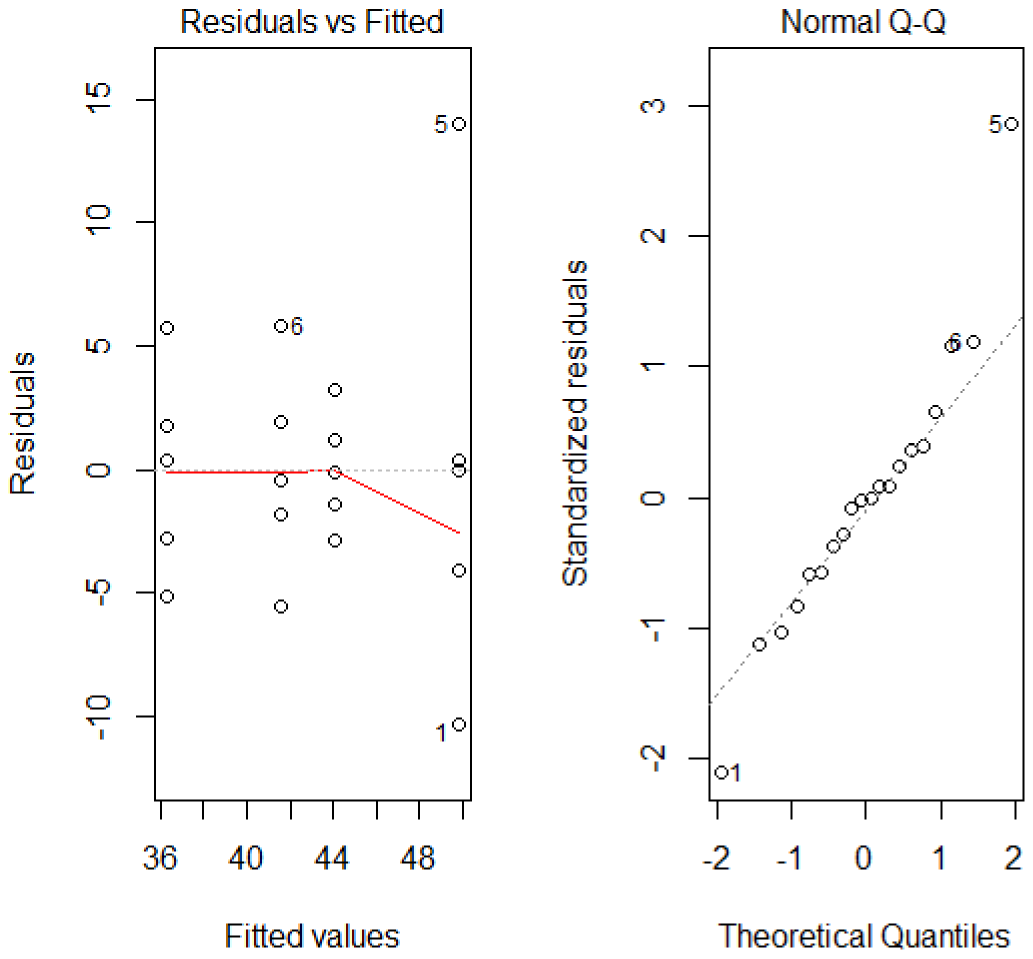
```
> summary.lm(SOLUB.AOV)
...

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



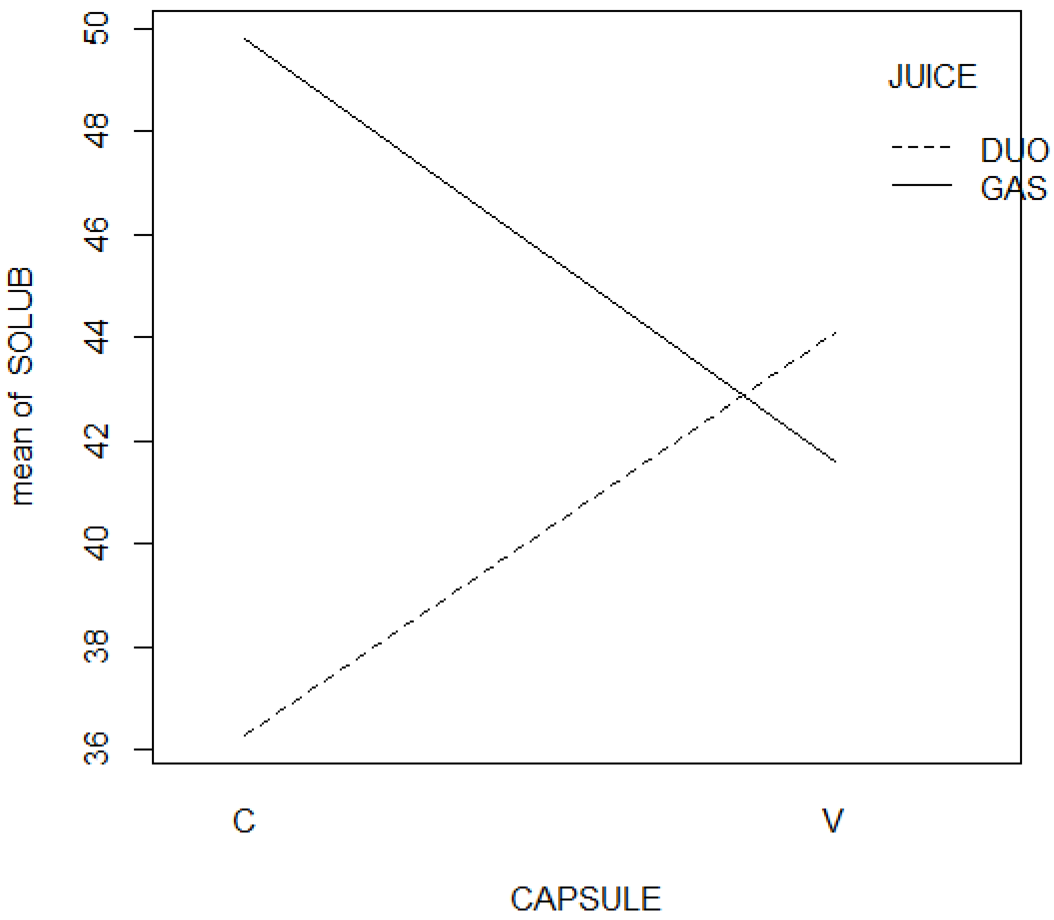
This graphic evaluates the overall residual and predicted values for the interaction effect.

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.

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

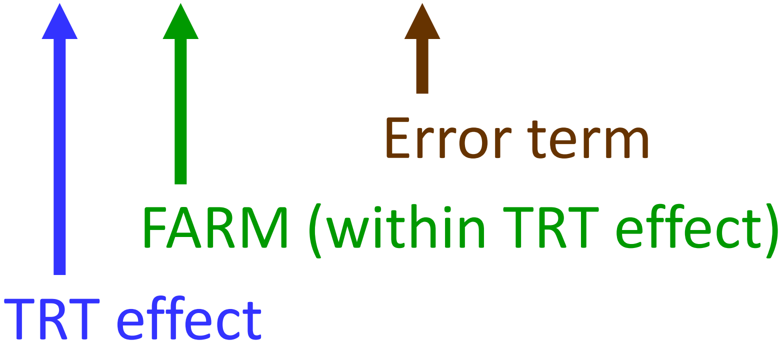
A less convenient way to present these data is as in our right. This array shows how an analysis similar to the previous one would have given many missing cells.

	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:

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



Error term

FARM (within TRT effect)

TRT effect

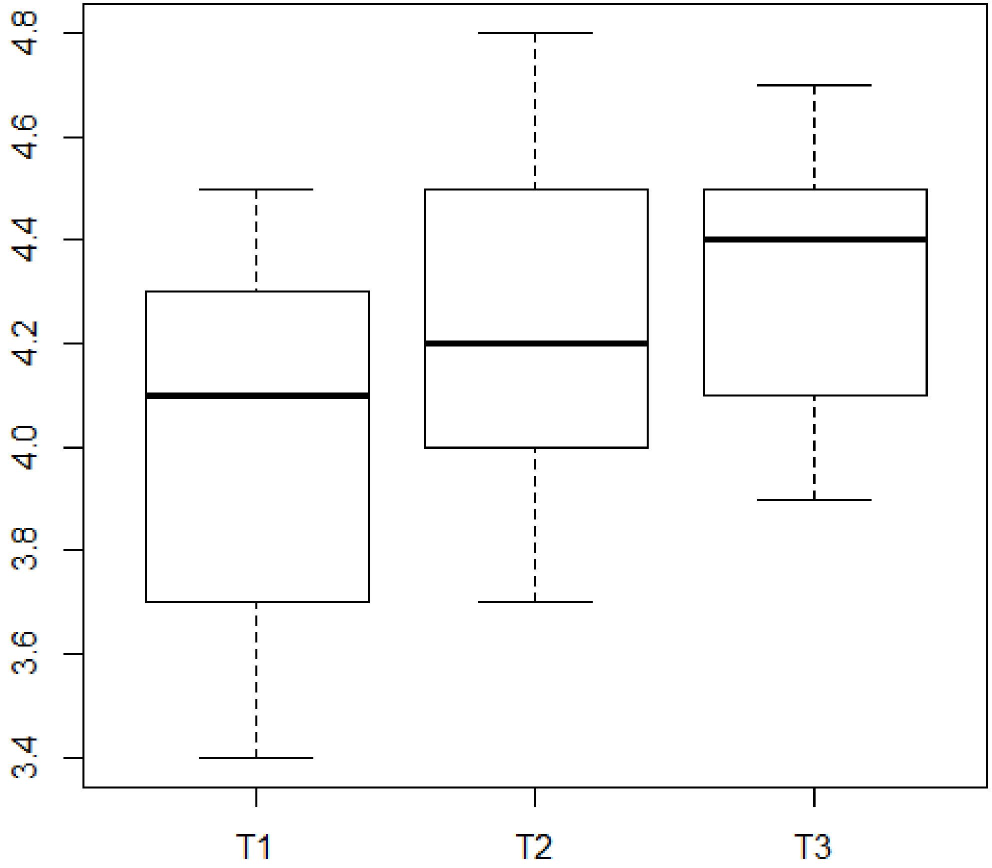
In some cases, we can be interested in contrasting the farm effects included in the model and then **farm is defined as a fixed effect**.

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

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

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

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.