*Curso de Formación de Personal Investigador*
*Usuario de Animales para Experimentación*

# Randomized Block Design.
# Factorial design

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# Learning objectives

- Define the concept of blocking as the third principle of the Experimental Design (Fisher) and the simplest model associated to it: the Randomized Complete Block Design (RCBD).
- Explain the reduction of the experimental noise as the main objective of including block effects.
- Establish a model describing RCBD and develop a R Commander program to contrast effects and compare means of the main factor (Tukey).
- Establish a criterion to test the superiority of RCBD on CRD.
- Define a Factorial Design with two main effects and their interaction.
- Develop a R Commander procedure to contrast the effects.
- List types of interaction effects and how to detect them, both numerically and through a graphic.
- Discuss the contrast of levels of the main effects and of the interaction effect (Tukey).

# Randomized Block Design

**Remember that blocking is one of the three principles of experimental design (the third one), in addition to replication (first) and random assignment of replicates to treatments (second).**

**Blocking has as a goal to reduce nuisance effects and to make "signal" stronger in relation to residual variation.**

We will analyse the kilocalories consumed by km in 3 types of activity, done by 8 people:

| | ACTIVITY | | |
|---|---|---|---|
| Person | Run | Walk | Pedal |
| 1 | 1.4 | 1.1 | 0.7 |
| 2 | 1.5 | 1.2 | 0.8 |
| 3 | 1.8 | 1.3 | 0,7 |
| 4 | 1.7 | 1.3 | 0.8 |
| 5 | 1.6 | 0.7 | 0.1 |
| 6 | 1.5 | 1.2 | 0.7 |
| 7 | 1.7 | 1.1 | 0.4 |
| 8 | 2.0 | 1.3 | 0.6 |

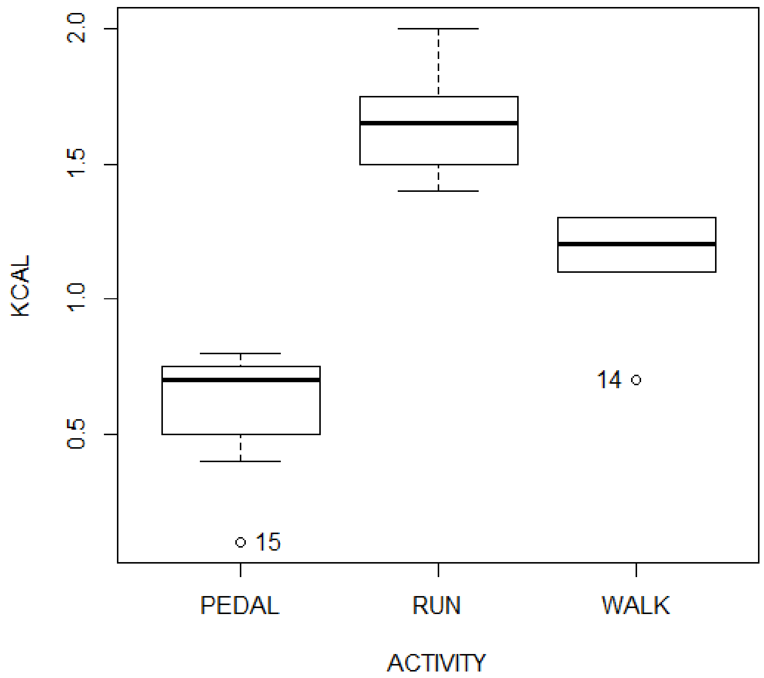
Milton, S. J. 2007. *Estadística para Biología y Ciencias de la salud*, 3ª ed. McGraw-Hill/ Interamericana de España.

# Exploring the distribution (boxplots)

Data: kcal

Graphs > Boxplot

Variable: KCAL; Plot by: ACTIVITY




No obvious violations of normality and homogeneity of variance: boxplots not asymmetrical and do not vary greatly in size, although two outliers can be observed

# First analysis (CRD)

We ignore the PERSON effect and analyse the data with CRD model.

Statistics > Fit models > Linear model

Model formula: KCAL ~ ACTIVITY

```
Coefficients:
                 Estimate Std. Error t value       Pr(>|t|)
(Intercept)        0.6000     0.0748   8.021 0.00000007892 ***
ACTIVITY[T.RUN]    1.0500     0.1058   9.926 0.00000000221 ***
ACTIVITY[T.WALK]   0.5500     0.1058   5.199 0.00003750129 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.2116 on 21 degrees of freedom
Multiple R-squared:  0.8244,  Adjusted R-squared:  0.8077
F-statistic:  49.3 on 2 and 21 DF,  p-value: 0.0000001168
```

In this case, the Residual standard error is 0.2116, and $R^2$ is 0.8244

# First analysis (CRD)

… and get an ANOVA table

Models > Hypothesis test > ANOVA table

```
Anova Table (Type II tests)

Response: KCAL
          Sum Sq Df F value         Pr(>F)
ACTIVITY  4.4133  2  49.298 0.00000001168 ***
Residuals 0.9400 21
```

The *p*-value of *F* tell us that the differences between activities are significant. However, we can refine this analysis

# **Second analysis:** Randomized Complete Block Design

Now we take into account the effect of the individual ($\varphi$): a **BLOCK**. The model is extended as follows:

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

In CRD (One-way ANOVA)

$$y_{ijk} = \mu + \tau_i + \underbrace{\varphi_j + \varepsilon'_{ijk}}$$

In RCBD. The error term of the previous model is partitioned into the individual effect and a new error (probably lower $\Rightarrow$ lower mean square error)

Furthermore, the PERSON effect can be considered a **random effect**, as we are not interested in the effect of each particular individual, but we have taken these individuals at random. Blocking aims at reducing **noise** (i.e., the residual mean square error).

# RCBD results

## Statistics > Fit models > Linear model

Model formula: KCAL ~ PERSON + ACTIVITY

```
Coefficients:
                  Estimate Std. Error t value        Pr(>|t|)
(Intercept)      5.333e-01  1.073e-01   4.972      0.000205 ***
PERSON[T.P2]     1.000e-01  1.357e-01   0.737      0.473320
PERSON[T.P3]     2.000e-01  1.357e-01   1.474      0.162634
PERSON[T.P4]     2.000e-01  1.357e-01   1.474      0.162634
PERSON[T.P5]    -2.667e-01  1.357e-01  -1.965      0.069550 .
PERSON[T.P6]     6.667e-02  1.357e-01   0.491      0.630824
PERSON[T.P7]    -4.121e-16  1.357e-01   0.000      1.000000
PERSON[T.P8]     2.333e-01  1.357e-01   1.720      0.107532
ACTIVITY[T.RUN]  1.050e+00  8.309e-02  12.636 0.00000000481 ***
ACTIVITY[T.WALK] 5.500e-01  8.309e-02   6.619 0.00001153120 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1662 on 14 degrees of freedom
Multiple R-squared:  0.9278,      Adjusted R-squared:  0.8813
F-statistic: 19.98 on 9 and 14 DF,  p-value: 0.000001657
```

In this case, the Residual standard error is 0.1662 (lower than in CRD) and $R^2$ is 0.93 (greater than in CRD)

# RCBD results (cont.)

… and get an ANOVA table

Models > Hypothesis test > ANOVA table

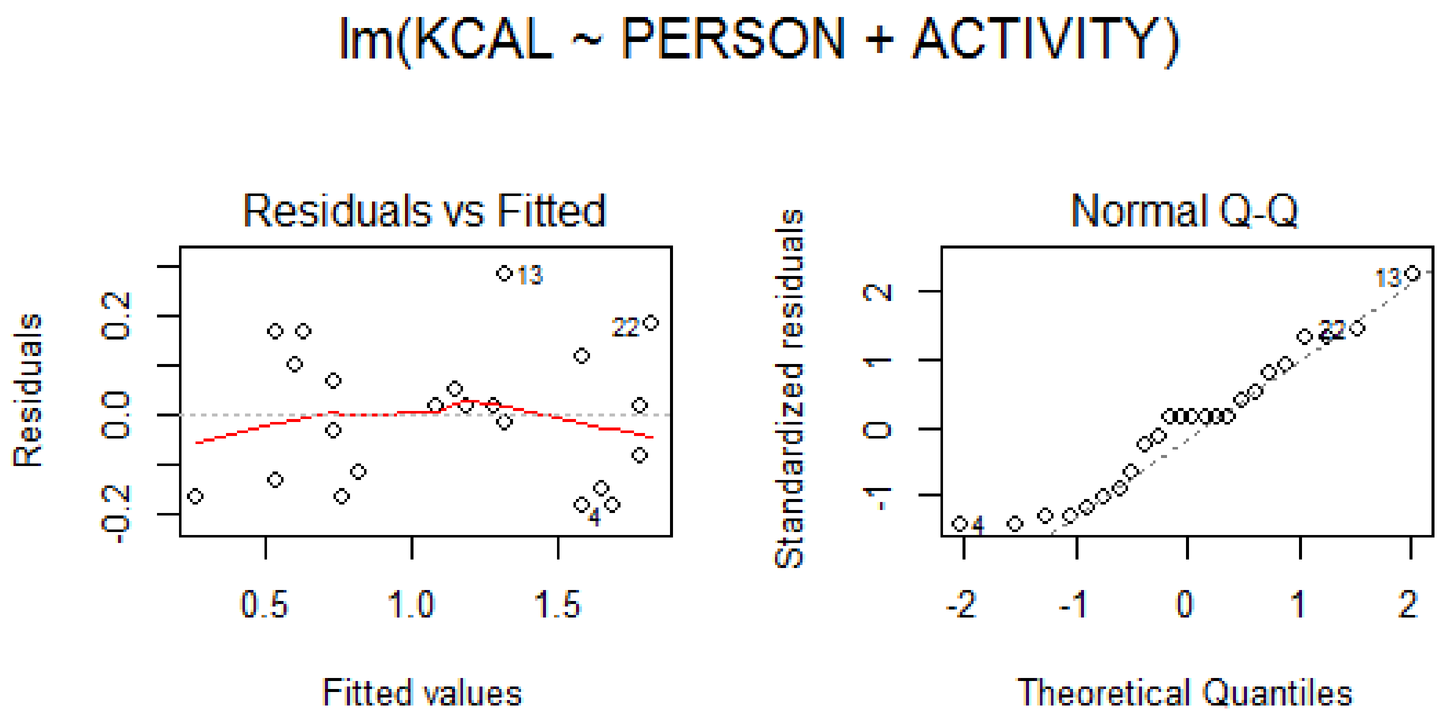
```
Anova Table (Type II tests)

Response: KCAL
          Sum Sq Df F value        Pr(>F)
PERSON    0.5533  7  2.8621       0.04462 *
ACTIVITY  4.4133  2 79.8966 0.00000002201 ***
Residuals 0.3867 14
```

Both PERSON and ACTIVITY are statistically significant

# RCBD diagnostics

Models > Graphs > Basic diagnostic plots




The residuals are fairly normal and do not suggest heterogeneity of variances

# RCBD contrast of means

```
> library(agricolae)
> HSD.test(LinearModel.2, "ACTIVITY", console=TRUE)

HSD Test for KCAL

ACTIVITY,  means
      KCAL       std r Min Max
PEDAL 0.60 0.2390457 8 0.1 0.8
RUN   1.65 0.1927248 8 1.4 2.0
WALK  1.15 0.2000000 8 0.7 1.3

Minimum Significant Difference: 0.2174827

Treatments with the same letter are not significantly different.

      KCAL groups
RUN   1.65      a
WALK  1.15      b
PEDAL 0.60      c
```

All three activities have a statistically significant different consumption of kilocalories

# Relative advantage of RCBD over CRD

We can compare the residual variance for designs with the same sample size. The *relative efficiency* in our case is:

$$\frac{\hat{\sigma}^2_{CRD}}{\hat{\sigma}^2_{RCBD}} = \frac{0.04476}{0.02762} = 1.62$$

The interpretation is that a CRD would require 62% more observations to obtain the same level of precision as a RCBD. The efficiency is not guaranteed to be greater than one. Only use blocking where there is some heterogeneity in the experimental units. The decision to block is a matter of judgment prior to the experiment. There is no guarantee that it will increase precision.

# Factorial design

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.

Milton, S. J. 2007. *Estadística para Biología y Ciencias de la salud*, 3ª ed. McGraw-Hill/Interamericana de España.

# Factorial design – 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**.

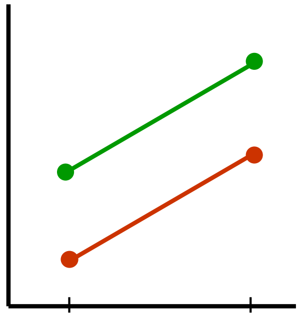
The model can include three or more main effects and their interactions (of two factors, three factors and higher levels).

# Interaction

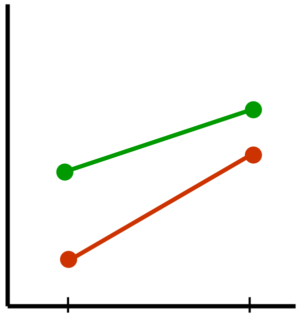
The dependence of the effect of one factor on the levels of another factor is called **interaction**.

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**.

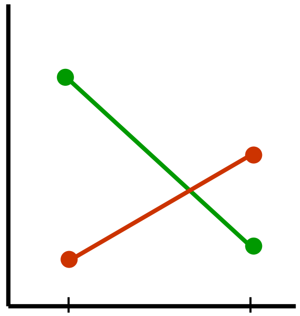
Any given combination of levels of factors may result in a positive or negative deviation from the expected value based on the means of the levels of the factors. If this deviation is positive we talk of **synergism**; if negative, **interference**. Both tend to magnify the interaction SS.



No interaction



Quantitative interaction



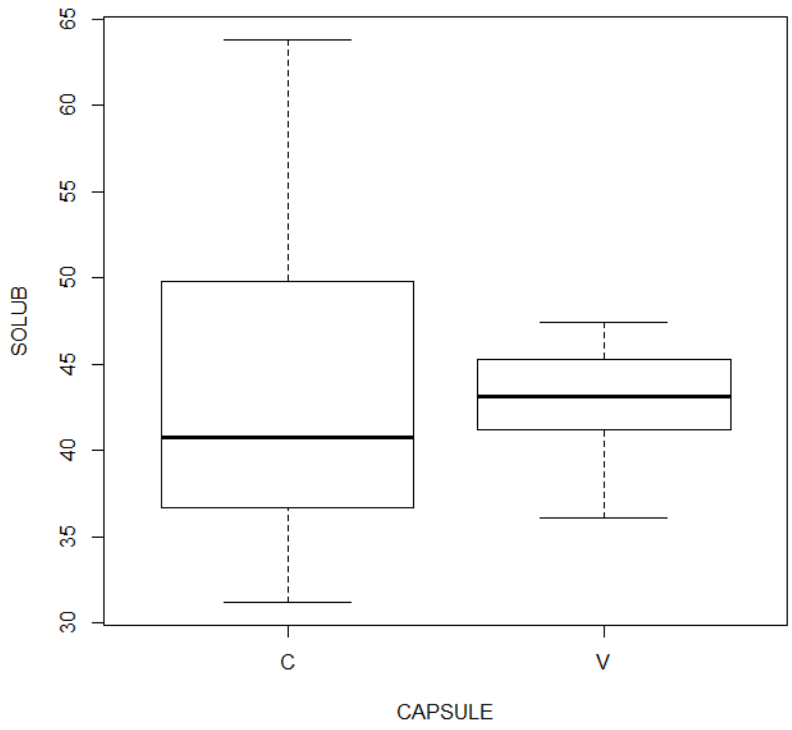
Qualitative interaction

# Factorial design – Boxplots

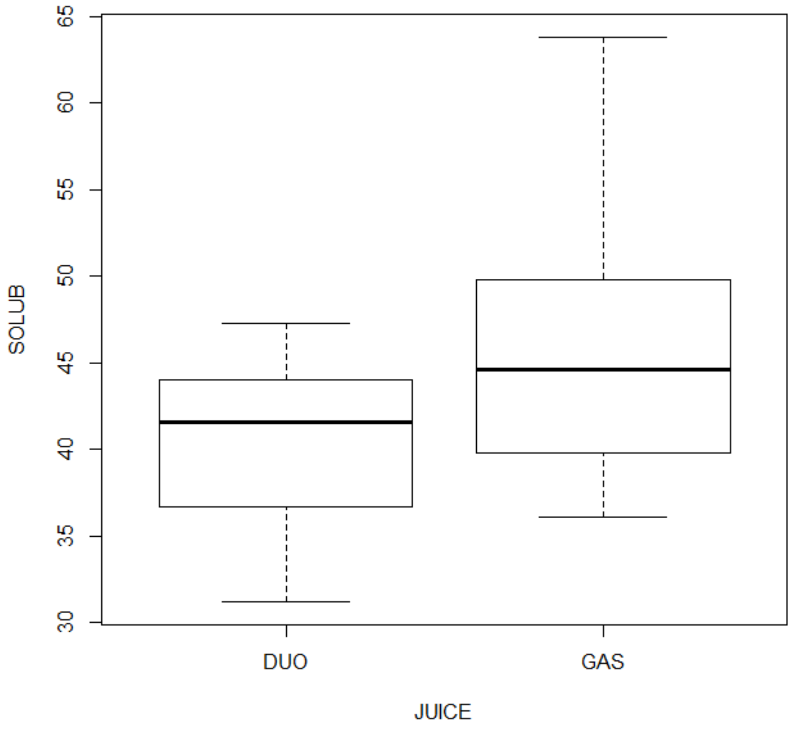
Data: solub

Graphs > Boxplot

Variable: SOLUB; Plot by: CAPSULE

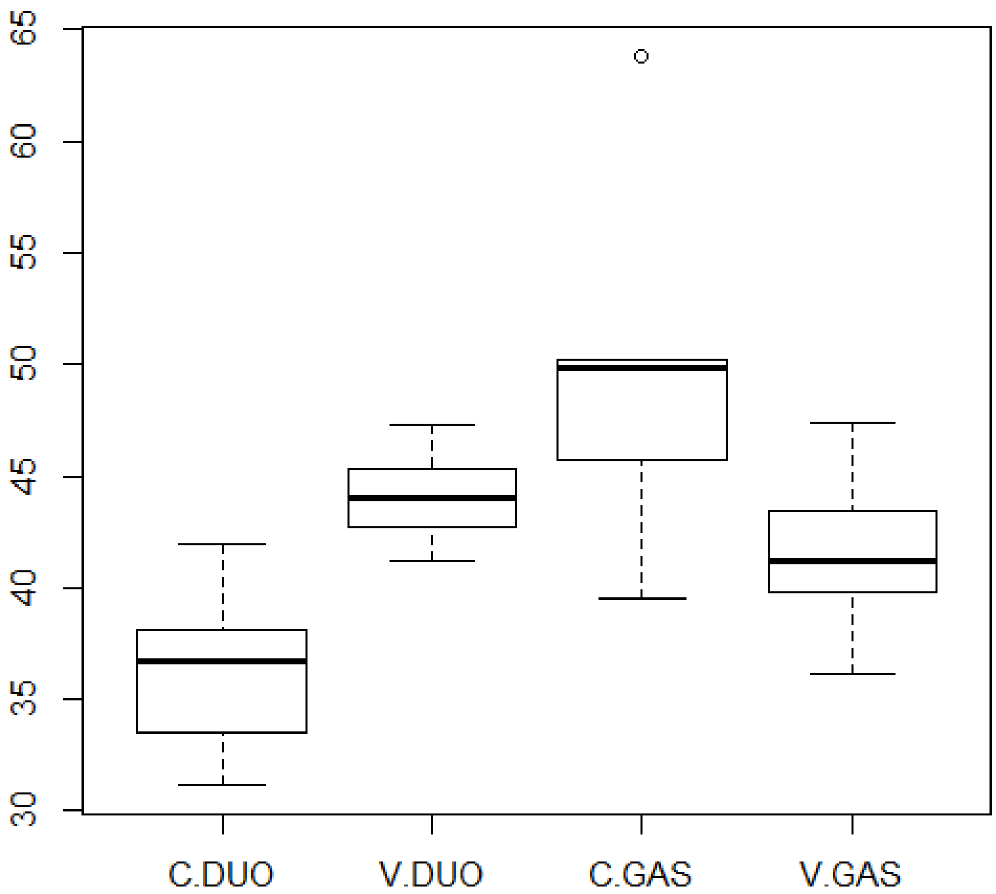



Plot by: JUICE



# Factorial design – Boxplot (cont.)

```
> boxplot(SOLUB~CAPSULE*JUICE, data=solub)
```



# Factorial design – Linear model analysis

Statistics > Fit models > Linear model

Model formula: SOLUB ~ CAPSULE * JUICE

Note that * between the two main effects is equivalent to define the sum of both main effects and the interaction effect

```
Coefficients:
                          Estimate Std. Error t value Pr(>|t|)    
(Intercept)                 36.300      2.454  14.792 9.42e-11 ***
CAPSULE[T.V]                 7.800      3.470   2.248  0.03906 *  
JUICE[T.GAS]                13.500      3.470   3.890  0.00130 ** 
CAPSULE[T.V]:JUICE[T.GAS]  -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 variability in solubility

# Factorial design – Anova table

Models > Hypothesis test > ANOVA table

```
Anova Table (Type II tests)

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

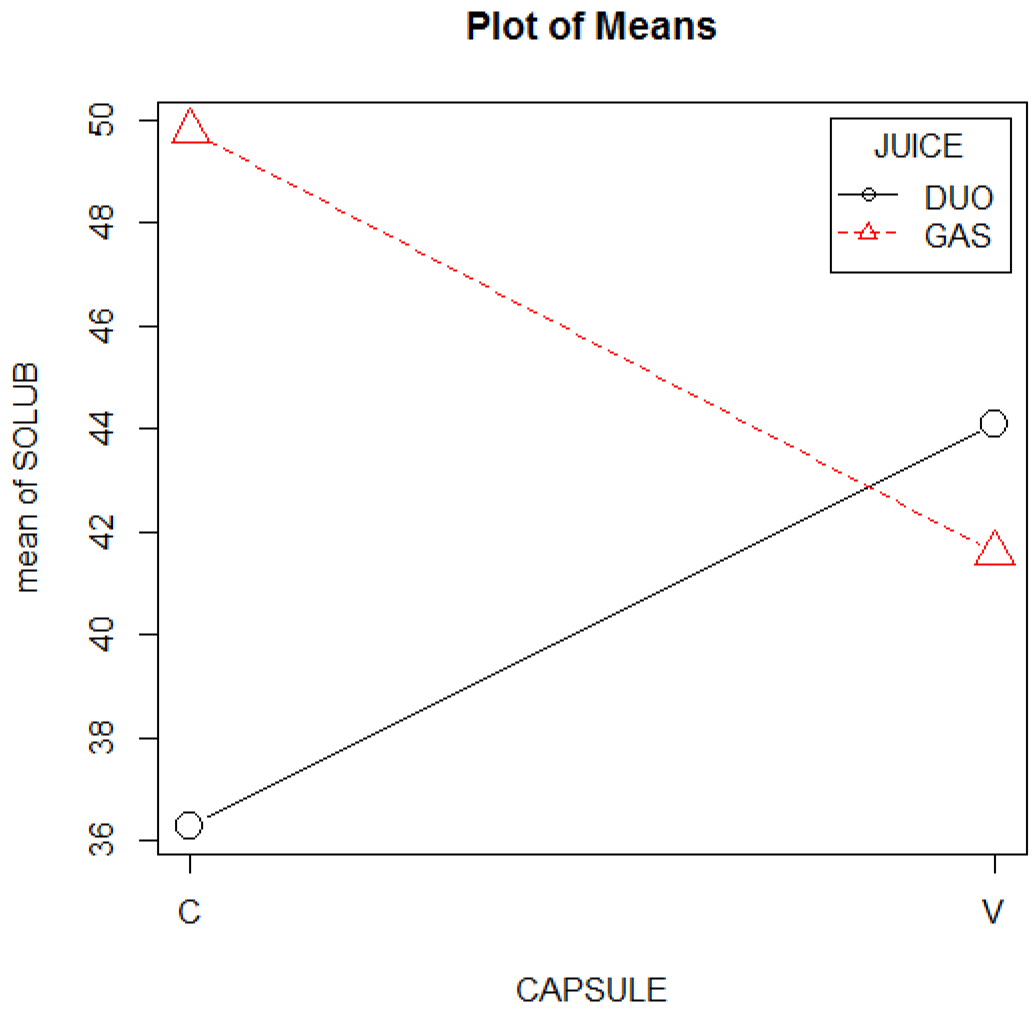
Juice and the interaction of Juice × Capsule are significant

# Factorial design – Graphical representation of interaction

## Graphs > Plot of means

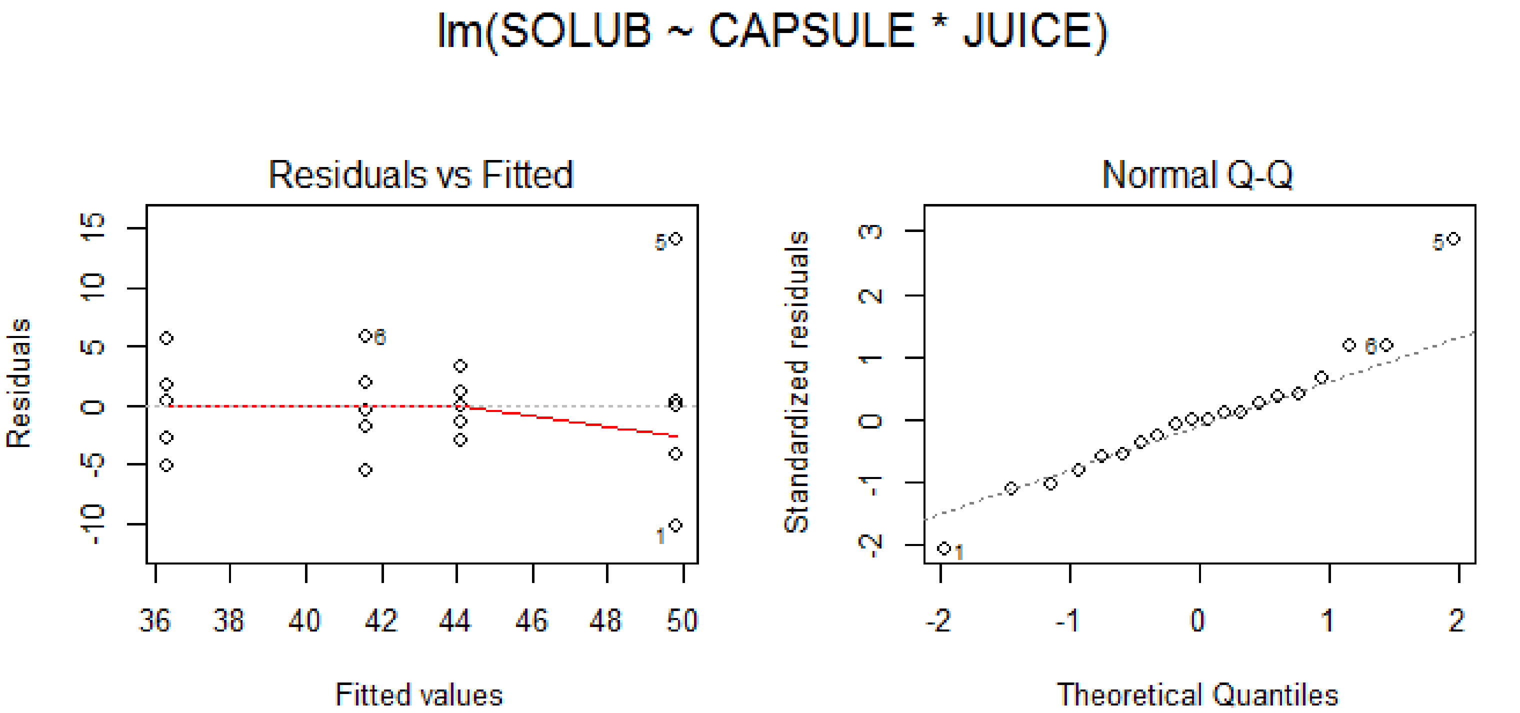
Factors: JUICE, CAPSULE; Response Variable: SOLUB

Options: No error bars, Top right



# Factorial design – Diagnostics

Models > Graphs > Basic diagnostic plots




No obvious violation of homogeneity of variance: no clear wedge shape in residuals

No obvious violation of normality: Q-Q plot of residuals is linear but for two residuals

# Factorial design – Comparison of means

```
> library(agricolae)
> HSD.test(LinearModel.3, c("CAPSULE","JUICE"), console=TRUE)

HSD Test for SOLUB

CAPSULE:JUICE,  means
       SOLUB      std r  Min  Max
C:DUO   36.3 4.175524 5 31.2 42.0
C:GAS   49.8 8.931125 5 39.5 63.8
V:DUO   44.1 2.348404 5 41.2 47.3
V:GAS   41.6 4.210107 5 36.1 47.4

Minimum Significant Difference: 9.929017

       SOLUB groups
C:GAS   49.8      a
V:DUO   44.1     ab
V:GAS   41.6     ab
C:DUO   36.3      b
```

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

We need to make only comparisons between combinations of levels