

Introduction to Statistics and Data Visualisation with R

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ANOVA



T-tests: summary

T-test in general

Used to compare means

One-sample t-test

Compare the mean of a sample to a given number

Two-sample t-test

Compare the means of two samples

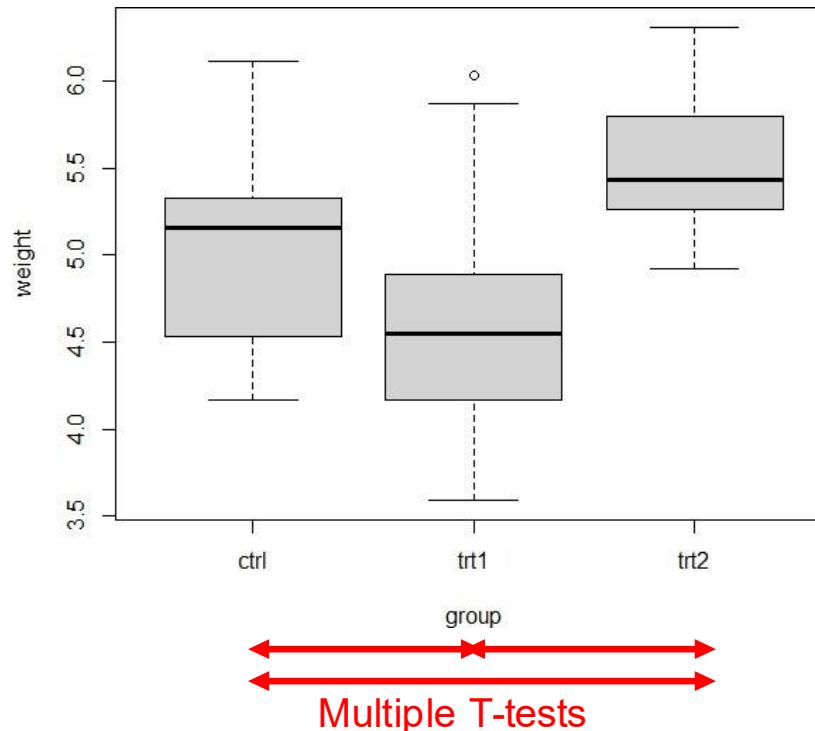
Paired t-test

Compare the difference between pairs of related data points

One or two groups

How to compare the mean of 3 groups ?

Example: What is the effect of treatment conditions on plant growth (weight) ?



How to compare the mean of 20 groups ?

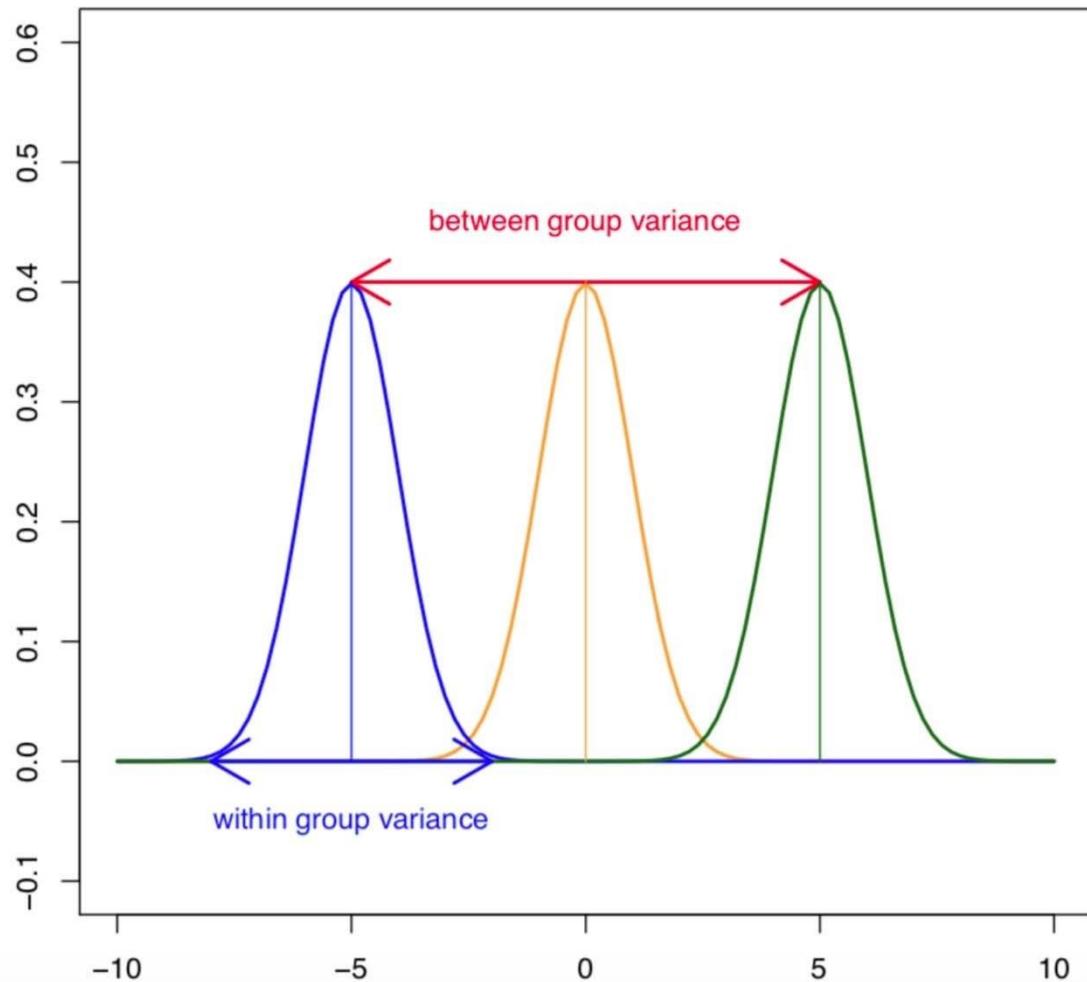
Multiple T-tests  Multiple testing correction !

Another solution ?

ANOVA = ANalysis Of Variance

allows to determine whether there are any statistically significant differences between the means of three or more independent groups

ANOVA – Schematic view



Within group variance = SS_{error}

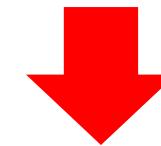
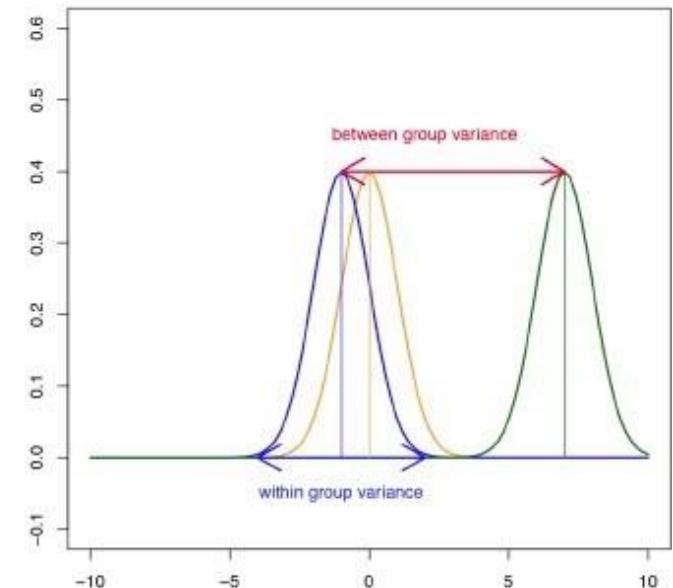
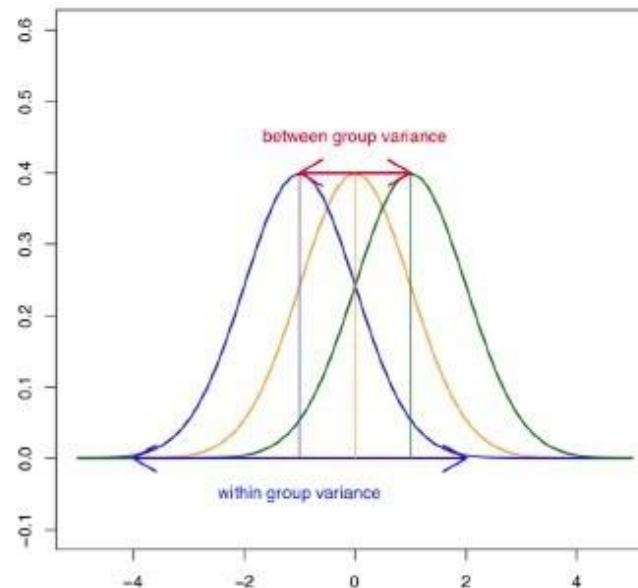
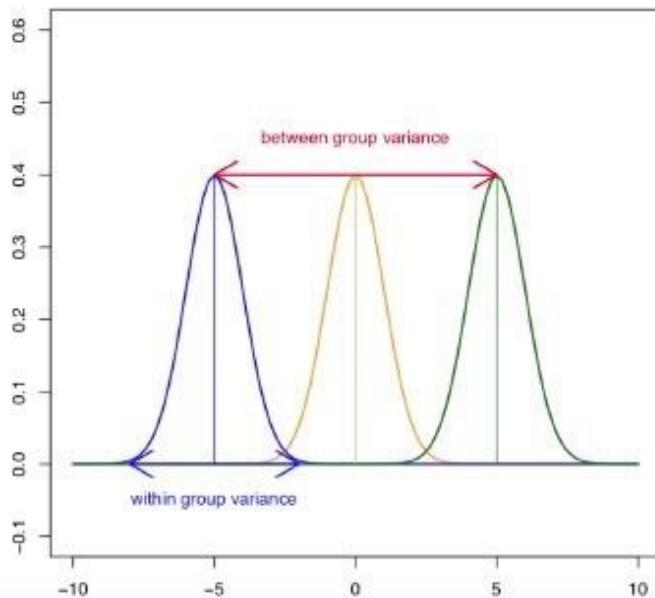
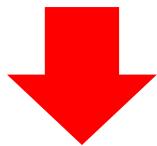
Assumption: $SS_{\text{error}}=SS_{\text{error}}=SS_{\text{error}}$

Between group variance = SS_{group}

$$SS_{\text{total}} = SS_{\text{group}} + SS_{\text{error}}$$

ANOVA – Schematic view

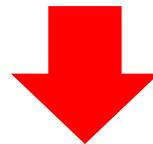
If $SS_{\text{group}} > SS_{\text{error}}$  at least two means are different



ANOVA – Hypothesis testing

- H_0 : all group means are equal
- H_1 : at least one mean is different
- A simple model formula in R with one factor is written as

plant weight ~ treatment
y ~ x



modeled by

ANOVA – in R

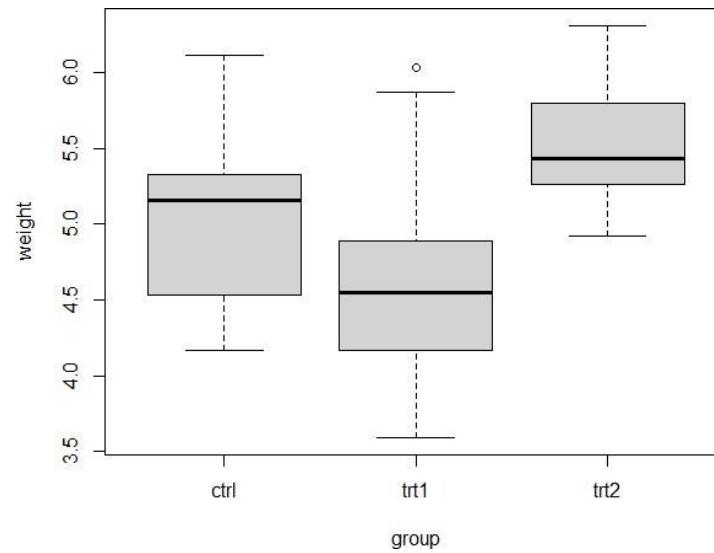
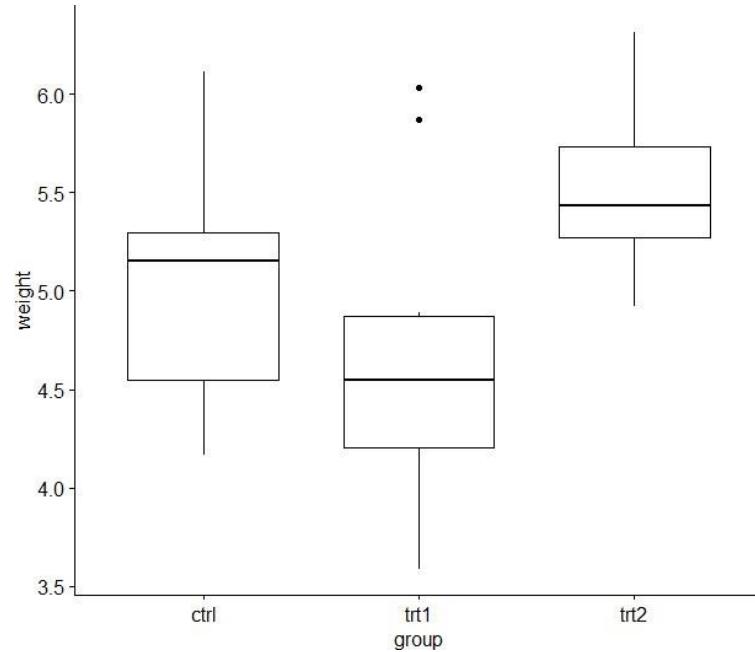
```
# read data
> PlantGrowth <- read.csv("PlantGrowth.csv", header = T)
> dim(PlantGrowth)
> levels(PlantGrowth$group)
> summary(PlantGrowth)

# if the levels are not automatically in the correct order, re-order them as follow:
> PlantGrowth <- PlantGrowth %>% reorder_levels(group, order = c("ctrl", "trt1",
"trt2"))

# compute some summary statistics (count, mean and sd) per group
> PlantGrowth %>% group_by(group) %>% get_summary_stats(weight, type = "mean_sd")
# A tibble: 3 x 5
  group variable     n   mean     sd
  <fct> <chr>    <dbl> <dbl> <dbl>
1 ctrl   weight     10   5.03  0.583
2 trt1   weight     10   4.66  0.794
3 trt2   weight     10   5.53  0.443
```

ANOVA – in R

```
# create a box plot of weight by group:  
> ggboxplot(PlantGrowth, x = "group", y = "weight")  
> boxplot(PlantGrowth$weight ~ PlantGrowth$group, xlab="group", ylab="weight")
```



ANOVA – in R

```
>anova.res <- aov(PlantGrowth$weight ~ PlantGrowth$group)
Call:
  aov(formula = PlantGrowth$weight ~ PlantGrowth$group)
```

Terms:

	PlantGrowth\$group	Residuals
Sum of Squares	3.76634	10.49209
Deg. of Freedom	2	27

Residual standard error: 0.6233746
Estimated effects may be unbalanced

```
> summary(anova.res)
      Df Sum Sq Mean Sq F value Pr(>F)
PlantGrowth$group    2   3.766   1.8832   4.846  0.0159 *
Residuals          27 10.492   0.3886
---
Signif. codes:  0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

ANOVA – in R

```
> summary(anova.res)
              Df Sum Sq Mean Sq F value Pr(>F)
PlantGrowth$group     2   3.766   1.8832   4.846 0.0159 *
Residuals            27  10.492   0.3886
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F ratio
Between groups (factor)	SSB	k-1	MSB=SSB/k-1	F=MSB/MSW
Within groups (error)	SSW	n-k	MSW=SSW/n-k	
Total	SST=SSB+SSW	n-1		

$$SSB = \sum_{j=1}^k n_j (\bar{X}_j - \bar{\bar{X}})^2$$

$$SSW = \sum_{j=1}^k \sum_{i=1}^{n_j} (X_{ij} - \bar{X}_j)^2$$

$$SST = \sum_{j=1}^k \sum_{i=1}^{n_j} (X_{ij} - \bar{\bar{X}})^2$$

ANOVA assumptions

- Independence of observations
- Equal variance

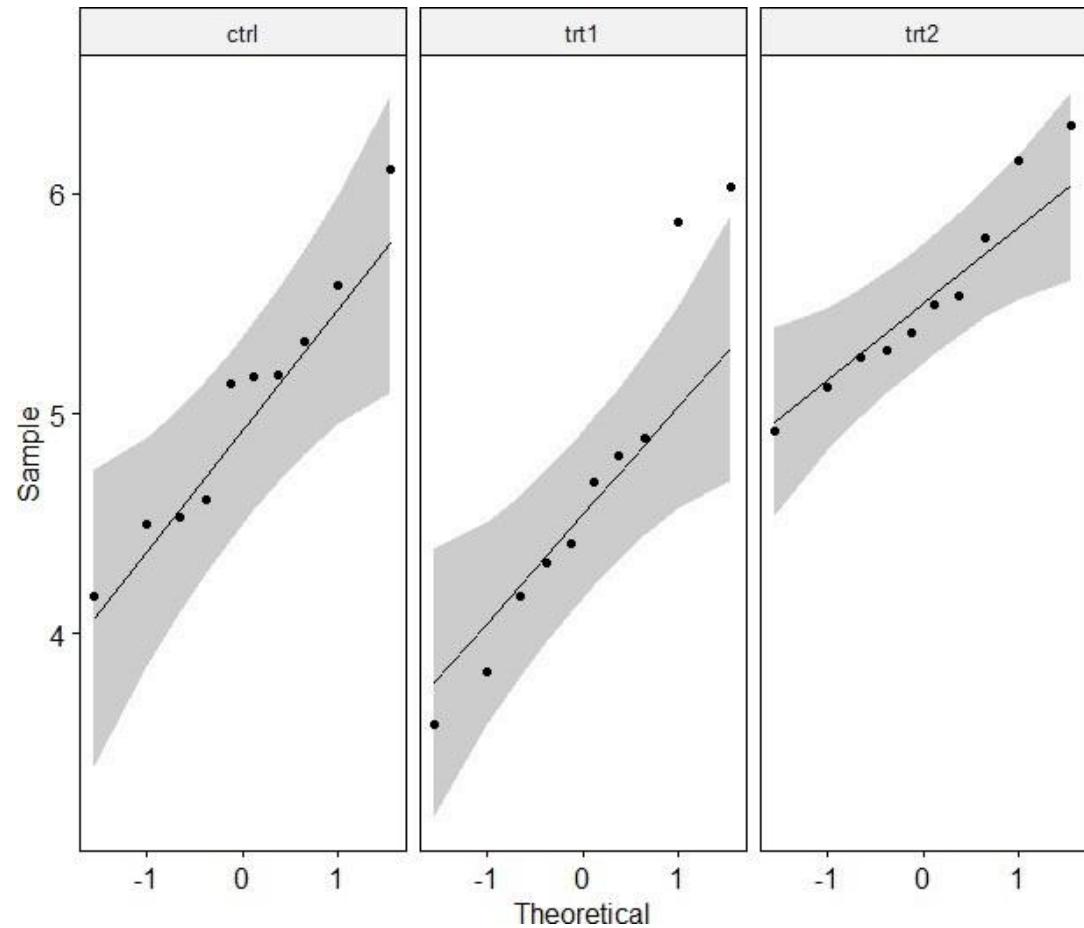
```
>PlantGrowth %>% levene_test(weight ~ group)
# A tibble: 1 x 4
  df1    df2 statistic     p
  <int> <int>    <dbl> <dbl>
1     2     27      1.12  0.341
```

$$W = \frac{\frac{n-k}{k-1} \sum_{i=1}^k n_i \left(\frac{1}{n_i} \sum_{j=1}^{n_i} |Y_{ij} - \bar{Y}_i| - \frac{1}{n} \sum_{i=1}^k \sum_{j=1}^{n_i} |Y_{ij} - \bar{Y}_i| \right)^2}{\sum_{i=1}^k \sum_{j=1}^{n_i} \left(|Y_{ij} - \bar{Y}_i| - \frac{1}{n_i} \sum_{j=1}^{n_i} |Y_{ij} - \bar{Y}_i| \right)^2} \sim F(k-1, n-k)$$

ANOVA assumptions

- Normal distribution

```
> PlantGrowth %>% group_by(group)  
%>% shapiro_test(weight)  
  
# A tibble: 3 x 4  
  group variable   statistic     p  
  <fct> <chr>       <dbl> <dbl>  
1 ctrl   weight      0.957  0.747  
2 trt1   weight      0.930  0.452  
3 trt2   weight      0.941  0.564  
  
> ggqqplot(PlantGrowth, "weight",  
  facet.by = "group")
```



Post-hoc tests

- A significant one-way ANOVA is generally followed up by Tukey post-hoc tests to perform multiple pairwise comparisons between groups

```
>tukey.res <- PlantGrowth %>% tukey_hsd(weight ~ group)
# A tibble: 3 x 9
  term   group1  group2  null.value  estimate  conf.low  conf.high p.adj p.adj.signif
* <chr> <chr>    <chr>        <dbl>      <dbl>      <dbl>      <dbl> <dbl> <chr>
1 group  ctrl     trt1         0       -0.371     -1.06      0.320 0.391 ns
2 group  ctrl     trt2         0        0.494     -0.197      1.19  0.198 ns
3 group  trt1     trt2         0        0.865      0.174      1.56  0.012 *
```

ANOVA is parametric

- ANOVA assumptions
 - Independence of observations
 - Equal variance
 - Normal distribution
- if the above assumptions are not met: non-parametric alternative:
Kruskal-Wallis test

```
> kruskal.res <- PlantGrowth %>% kruskal_test(weight ~ group)
> kruskal.res
# A tibble: 1 x 6
  .y.      n statistic    df      p method
* <chr> <int>     <dbl> <int>    <dbl> <chr>
1 weight     30      7.99     2  0.0184 Kruskal-Wallis
```

Two-way ANOVA

- Example: the combined effect of treatment type and concentration on the growth (weight) of plants

Concentration	Treatment type		
	Control	Treatment 1	Treatment 2
Low			
High			

ANOVA – Hypothesis testing

- A model formula in R with x factors is written as

$$y \sim x_1 + x_2 + x_3$$

Response ~ predictors

- Some useful symbols
 - + add more variables
 - leave out variables
 - :
interaction between two terms
- *
include the terms and the interactions $a * b = a + b + a : b$ - n adds all terms and all interactions up to order n
- I () include a mathematical expression

Two-way ANOVA

- Example: the combined effect of treatment type and concentration on the growth (weight) of plants

Concentration	Treatment type		
	Control	Treatment 1	Treatment 2
Low			
High			

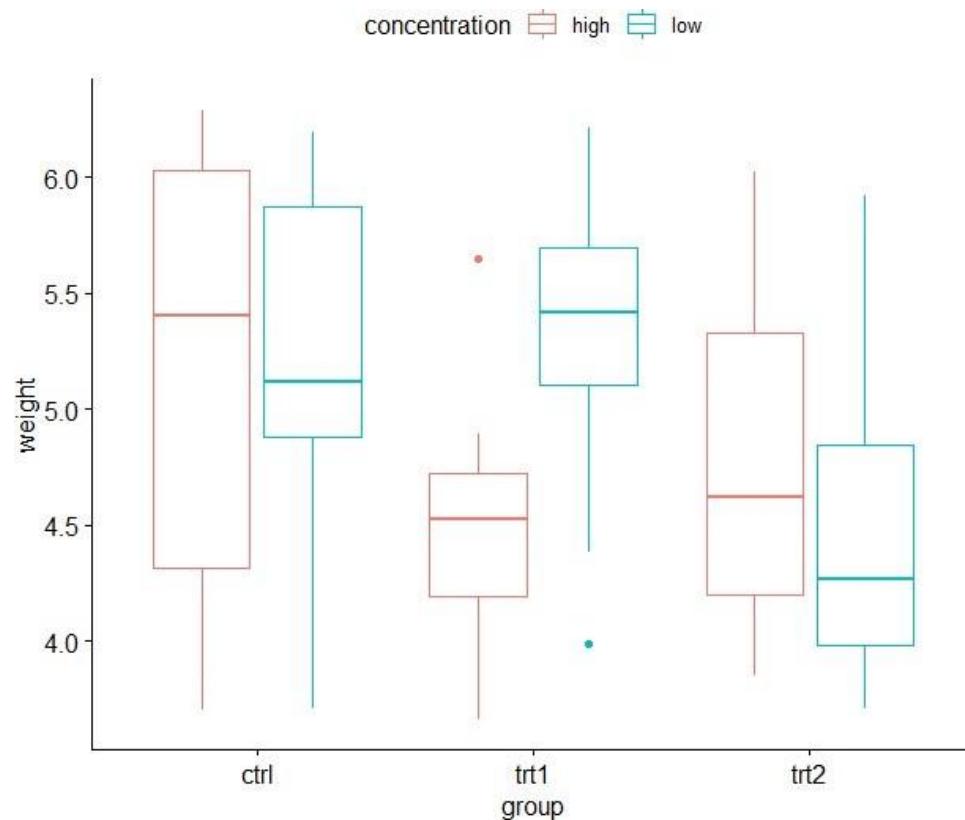
Plant growth ~ treatment type * concentration

ANOVA – in R

```
# compute some summary statistics (count, mean and sd) per group
>PlantGrowth_new %>% group_by(group, concentration) %>%
get_summary_stats(weight, type = "mean_sd")
# A tibble: 6 x 6
  group concentration variable     n   mean    sd
  <chr> <chr>          <chr> <dbl> <dbl> <dbl>
1 ctrl   high            weight    10  5.16  1.00
2 ctrl   low             weight    10  5.24  0.755
3 trt1   high            weight    10  4.51  0.552
4 trt1   low             weight    10  5.30  0.69
5 trt2   high            weight    10  4.77  0.745
6 trt2   low             weight    10  4.55  0.775
```

ANOVA – in R

```
# visualization  
> ggboxplot(PlantGrowth_new, x = "group", y = "weight", color = "concentration")
```



ANOVA – in R – check assumptions

- Independence of observations
- Equal variance

```
>PlantGrowth_new %>% levene_test(weight ~ group*concentration)
# A tibble: 1 x 4
  df1    df2 statistic     p
  <int> <int>    <dbl> <dbl>
1      5      54     0.898 0.489
```

ANOVA – in R – check assumptions

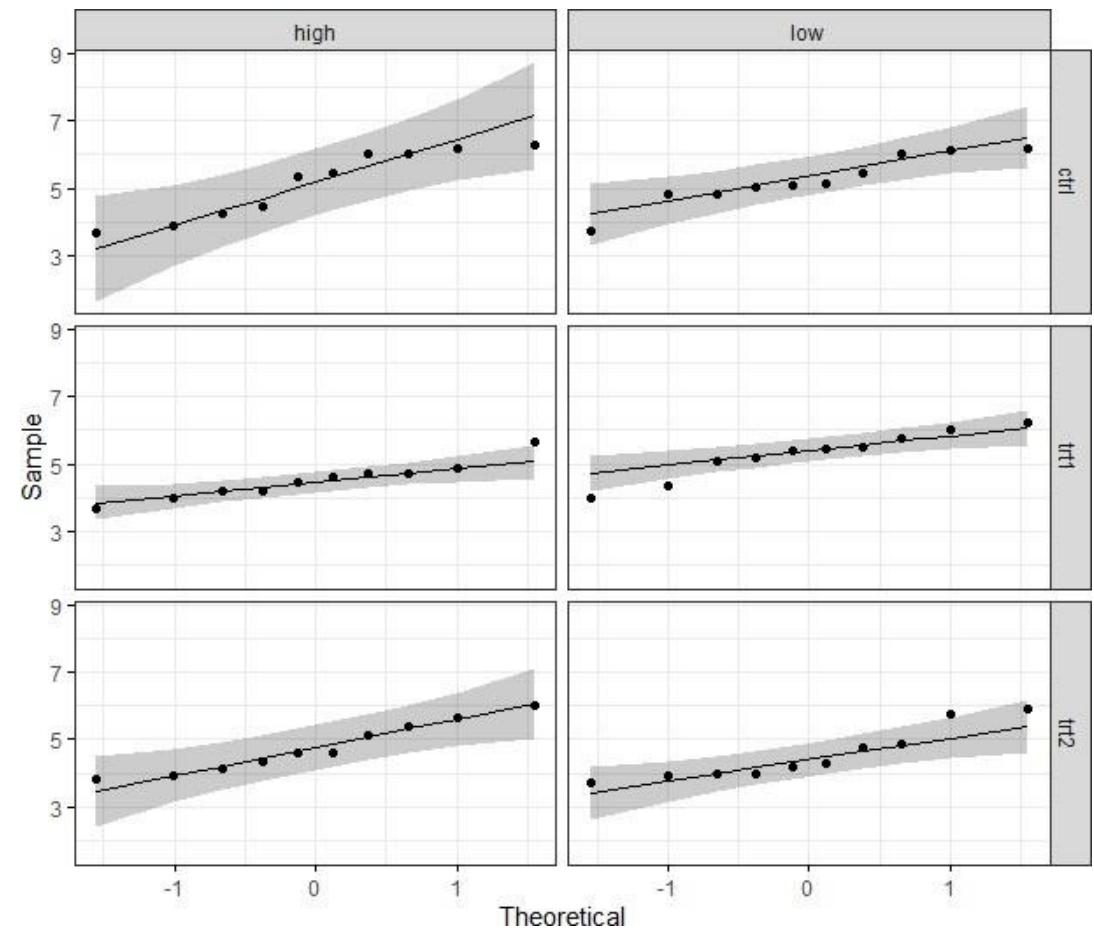
- Normal distribution

```
> PlantGrowth_new %>% group_by(group, concentration) %>% shapiro_test(weight)
# A tibble: 6 x 5
  group  concentration  variable   statistic      p
  <chr> <chr>          <chr>       <dbl>    <dbl>
1 ctrl   high           weight     0.883  0.143
2 ctrl   low            weight     0.914  0.313
3 trt1  high           weight     0.963  0.817
4 trt1  low            weight     0.941  0.562
5 trt2  high           weight     0.943  0.585
6 trt2  low            weight     0.867  0.093
```

ANOVA – in R – check assumptions

- Normal distribution

```
>ggqqplot(PlantGrowth_new,  
"weight", ggtheme = theme_bw()) +  
facet_grid(group ~ concentration)
```



ANOVA – in R

```
>anova.res <- aov(PlantGrowth_new$weight ~ PlantGrowth_new$group *  
PlantGrowth_new$concentration)  
> summary(anova.res)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
PlantGrowth_new\$group	2	2.980	1.4898	2.548	0.0876 .
PlantGrowth_new\$concentration	1	0.700	0.6998	1.197	0.2788
PlantGrowth_new\$group:PlantGrowth_new\$concentration	2	2.734	1.3668	2.338	0.1063
Residuals	54	31.575	0.5847		

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

ANOVA – in R

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F ratio
Factor A	SSA	a-1	MSA = SSA/(a-1)	MSA/MSE
Factor B	SSB	b-1	MSB = SSB/(b-1)	MSB/MSE
Interaction	SSAB	(a-1)(b-1)	SSAB = MSAB/(a-1)(b-1)	MSAB/MSE
Error	SSE	ab(n _{ij} -1)	SSE = MSE/(ab(n _{ij} -1))	
Total	SST	n-1		

X_{ijk} : value of kth observation of level i of factor A and level j of factor B

n_i : number of observations of level i of factor A

n_j : number of observations of level j of factor B

n_{ij} : number of observations of level i of factor A and level j of factor B

$$SSA = \sum_{i=1}^a n_i (\bar{X}_i - \bar{\bar{X}})^2$$

$$SSB = \sum_{j=1}^b n_j (\bar{X}_j - \bar{\bar{X}})^2$$

$$SSAB = \sum_{i=1}^a \sum_{j=1}^b n_{ij} (\bar{X}_{ij} - \bar{X}_i - \bar{X}_j + \bar{\bar{X}})^2$$

$$SSE = \sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^{n_{ij}} (X_{ijk} - \bar{X}_{ij})^2$$

$$SST = \sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^{n_{ij}} (X_{ijk} - \bar{\bar{X}})^2$$

Confidence intervals

Confidence intervals

- Confidence interval is related to the p-value.
- It is a measure of the study's precision.
- P-value answers the question:

"Is there a statistically significant difference between the two treatments ?"

- The point estimate and its confidence interval answer the questions:

"What is the size of that treatment difference?"

"How precisely did this trial determine or estimate the treatment difference?"

Confidence intervals - representation

- Width of a confidence interval:



Confidence Limits: The upper and lower end points of the confidence interval.

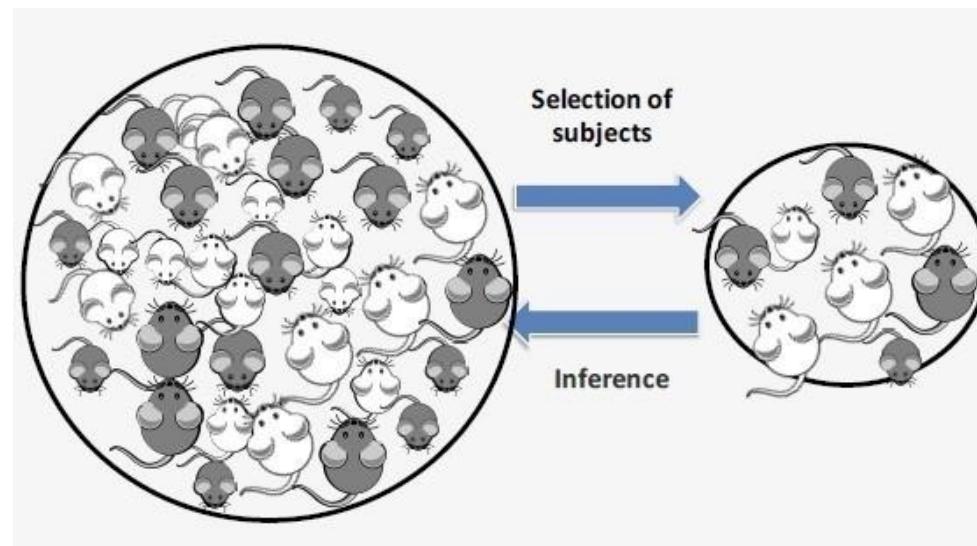
- A narrow CI implies high precision
- A wide CI implies poor precision (usually due to inadequate sample size)

Confidence intervals – computation

- $CI = (\text{Sample statistic}) \pm [(\text{critical value}) \times (\text{Sampling variability measure})]$
 - Sample statistic: observed magnitude of effect or association (e.g., odds ratio, risk ratio, difference in mean)
 - Critical value: reflects on how confident you want to be, related to the statistics and to your level of confidence ($1.0 - \alpha$). The latter is usually expressed as a percentage (e.g. 90%, 95% or 99%). At 95 % the t-statistics critical value is 1.96 for example.
 - Sampling variability: a measure of how high the sampling variability is. Ex: Standard error (S.E.) of the estimate is a measure of variability

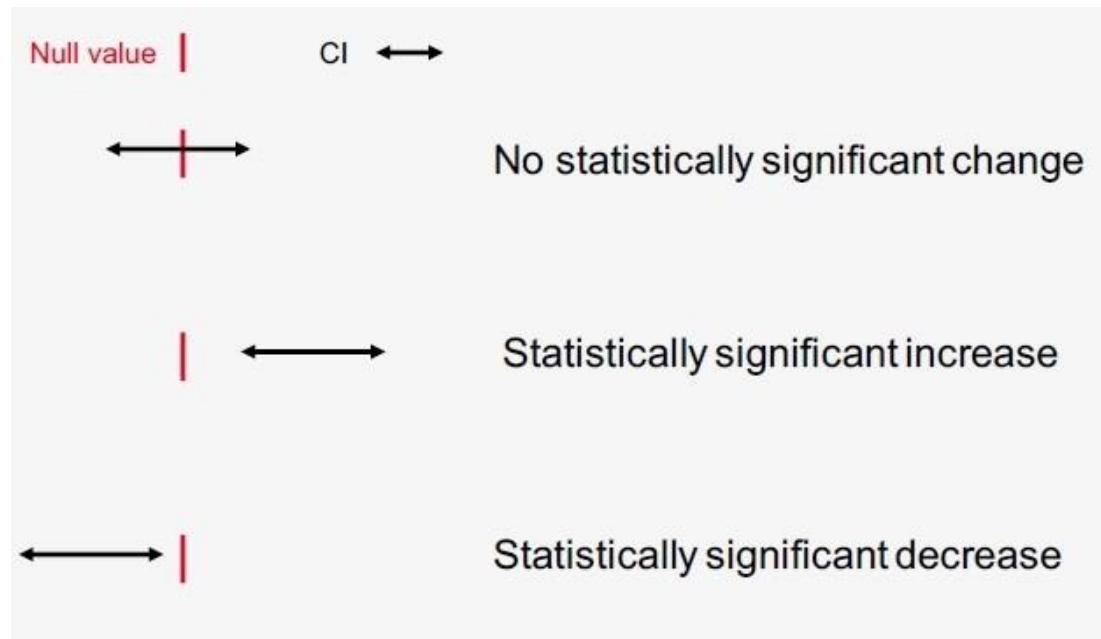
Confidence intervals – interpretation

- 95% C.I. means that true estimate of effect (ex: difference in mean, risk, rate) lies within 1.96 "standard errors" of the population mean 95 times out of 100 (given some assumptions).



Confidence intervals – interpretation

- If the 95% confidence interval does **NOT** include the null value, then we declare a “**statistically significant**” association.
- If the 95% confidence interval includes the null value, then the test result is “**not statistically significant**.”



Confidence intervals – interpretation

- Interpretation of C.I. for means: does the interval include 0 ?
- Interpretation of C.I. for ratio: does the interval include 1 ?
- Connection between P-values and C.I.s (they are mathematically connected!)
 - If a 95% CI includes the null effect, the Pvalue is > 0.05 (and we would fail to reject the null hypothesis)
 - If the 95% CI excludes the null effect, the Pvalue is < 0.05 (and we would reject the null hypothesis)

Confidence intervals – interpretation

<u>Exposure:</u>	alcohol intake (high versus low)	Women with high alcohol intake are 1.32 times (or 32%) more likely to develop breast cancer compared to women with low alcohol intake. However, we are 95% confident that the true value (risk) of the population lies between 0.87 and 1.98 => not significant !
<u>Outcome:</u>	Incidence of breast cancer	
<u>Risk Ratio:</u>	1.32 (point estimate)	
<u>p-value:</u>	0.14 (not statistically significant)	
<u>95% C.I.:</u>	0.87 - 1.98	

