UNIVERSITÉ DU LUXEMBOURG

LUXEMBOURG INSTITUTE OF HEALTH
RESEARCH DEDICATED TO LIFE
Multiomics Data Science

BIOSTATISTICS

Lecture 10

Analysis of Variance (ANOVA)

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OUTLINE

Lecture 10

- **Introduction to ANOVA**
  - why ANOVA
  - shoe experiment
  - assumptions with ANOVA
- **Single-factor ANOVA**
- **Multi-factor ANOVA**
- **Experimental design**
  - randomized design
  - block design

INTRODUCTION TO ANOVA

Why ANOVA?

Means for more than 2 populations
We have measurements for 5 conditions. Are the means for these conditions equal?

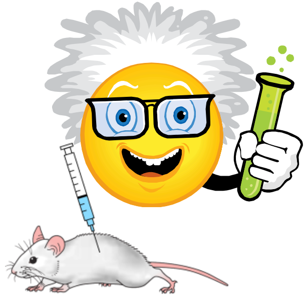
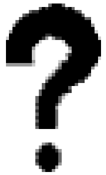
Validation of the effects
We assume that we have several factors affecting our data. Which factors are most significant? Which can be neglected?

ANOVA
example from Partek™

If we would use pairwise comparisons, what will be the probability of getting error?

Number of comparisons: $C_2^5 = \frac{5!}{2!3!} = 10$

Probability of an error: $1 - (0.95)^{10} = 0.4$

http://easylink.playstream.com/affymetrix/ambsymposium/partek_08.wvx

INTRODUCTION TO ANOVA

Example

As part of a long-term study of individuals 65 years of age or older, sociologists and physicians at the Wentworth Medical Center in upstate New York investigated the relationship between geographic location and depression. A sample of 60 individuals, all in reasonably good health, was selected; 20 individuals were residents of Florida, 20 were residents of New York, and 20 were residents of North Carolina. Each of the individuals sampled was given a standardized test to measure depression. The data collected follow; higher test scores indicate higher levels of depression.

Q: Is the depression level same in all 3 locations?

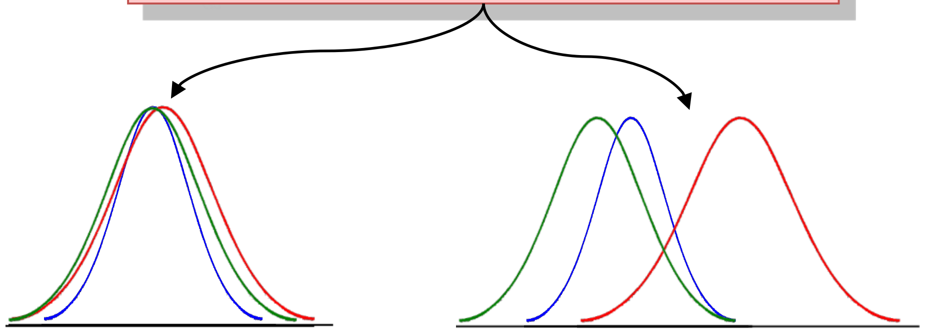
`depression.xls`

1. Good health respondents

Florida	New York	N. Carolina
3	8	10
7	11	7
7	9	3
3	7	5
8	8	11
8	7	8
…	…	…

H_0: $\mu_1 = \mu_2 = \mu_3$

H_a: not all 3 means are equal

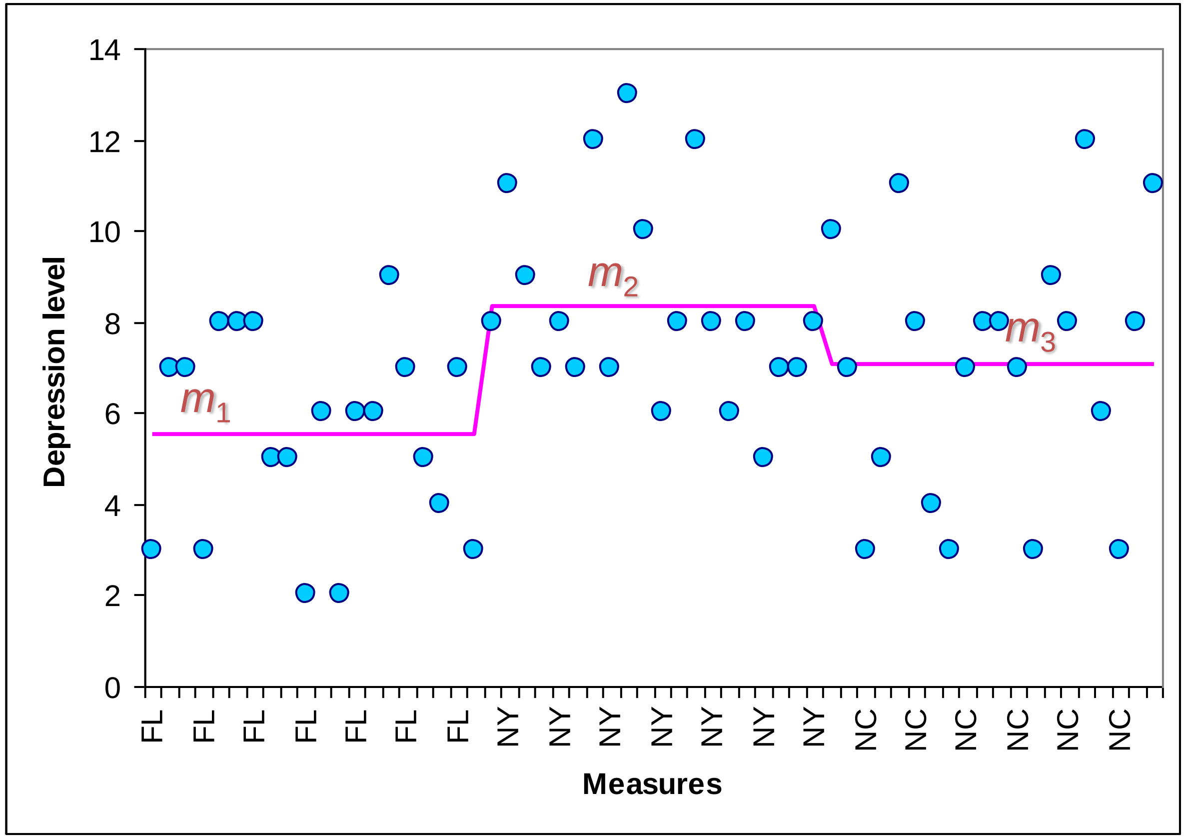


INTRODUCTION TO ANOVA

Meaning

H_0: $\mu_1 = \mu_2 = \mu_3$

H_a: not all 3 means are equal



INTRODUCTION TO ANOVA

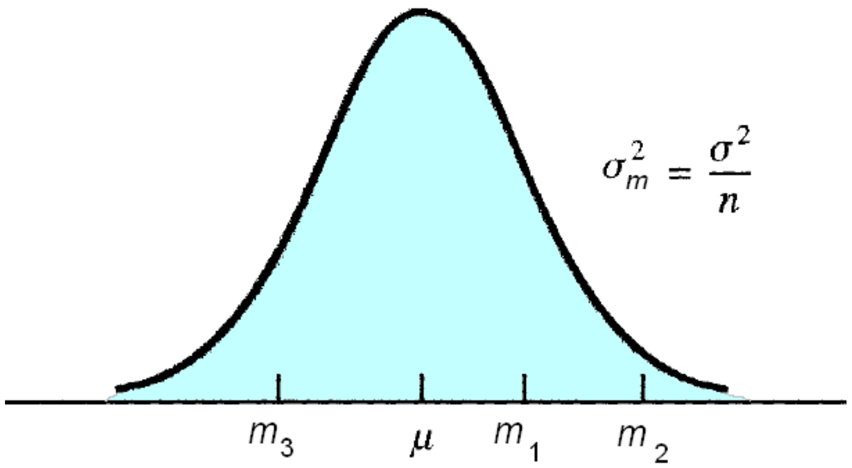
Assumptions for ANOVA

Assumptions for Analysis of Variance

1. For each population, the response variable is **normally distributed**

2. The variance of the respond variable, denoted as **σ^2 is the same** for all of the populations.

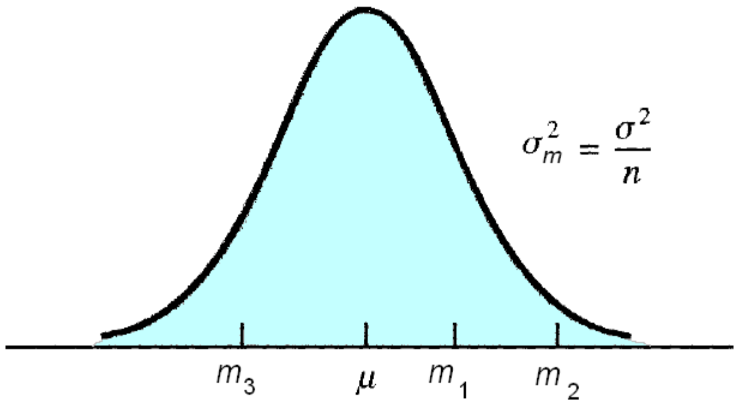
3. The observations must be **independent.**



INTRODUCTION TO ANOVA

Some Calculations

Parameter	Florida	New York	N. Carolina
m=	5.55	8.35	7.05
overall mean=	6.98333		
var=	4.5763	4.7658	8.0500



Let's estimate the variance of sampling distribution. If H_0 is true, then all m_i belong to the same distribution

$$\sigma_m^2 = \frac{\sum_{i=1}^{k}(m_i - \overline{m})^2}{k-1} = \frac{(5.55-6.98)^2 + (8.35-6.98)^2 + (7.05-6.98)^2}{3-1} = 1.96$$

$$\sigma^2 = n\sigma_m^2 = 20 \times 1.96 = 39.27$$

– this is called between-treatment estimate, works only at H_0

At the same time, we can estimate the variance just by averaging out variances for each populations:

– this is called within-treatment estimate

$$\sigma^2 = \frac{\sum_{i=1}^{k}\sigma_i^2}{k} = \frac{4.58 + 4.77 + 8.05}{3} = 5.8$$

Does between-treatment estimate and within-treatment estimate give variances of the same “population”?

SINGLE-FACTOR ANOVA

Theory

H_0: $\mu_1 = \mu_2 = \ldots = \mu_k$

H_a: not all k means are equal

Means for treatments

$$m_j = \frac{\sum_{i=1}^{n_j} x_{ij}}{n_j}$$

Variances treatments

$$s_j^2 = \frac{\sum_{i=1}^{n_j} (x_{ij} - m_j)^2}{n_j - 1}$$

Total mean

$$\overline{m} = \frac{\sum_{j=1}^{k} \sum_{i=1}^{n_j} x_{ij}}{n_T}$$

$$n_T = n_1 + n_2 + \cdots + n_k$$

due to treatment

Sum squares

$$SSTR = \sum_{j=1}^{k} n_j (m_j - \overline{m})^2$$

Mean squares, $\sigma_{beetween}^2$

$$MSTR = \frac{SSTR}{k-1}$$

due to error

Sum squares

$$SSE = \sum_{j=1}^{k} (n_j - 1) s_j^2$$

Mean squares, σ_{within}^2

$$MSE = \frac{SSE}{n_T - k}$$

Test of variance equality

$$F = \frac{MSTR}{MSE}$$

p-value for the treatment effect

$$p - value$$

SINGLE-FACTOR ANOVA

The Main Equation

Total sum squares

$$SST = \sum_{j=1}^{k}\sum_{i=1}^{n_j}\left(x_{ij} - \overline{m}\right)^2$$

SS due to treatment

$$SSTR = \sum_{j=1}^{k} n_j\left(m_j - \overline{m}\right)^2$$

$$SST = SSTR + SSE$$

SS due to error

$$SSE = \sum_{j=1}^{k}\left(n_j - 1\right)s_j^2$$

Total variability of the data include variability due to treatment and variability due to error

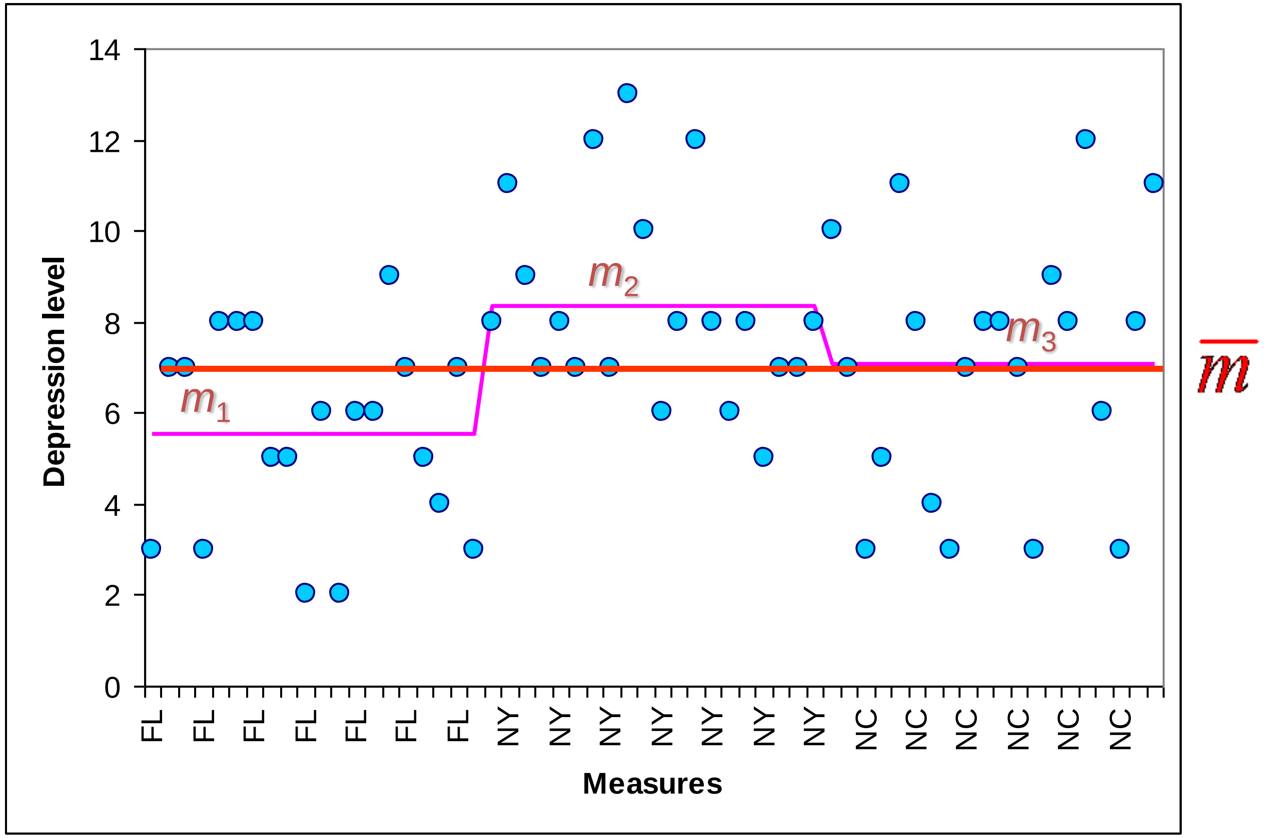
$$d.f.(SST) = d.f.(SSTR) + d.f.(SSE)$$
$$n_T - 1 = \left(k - 1\right) + \left(n_T - k\right)$$

Partitioning

The process of allocating the total sum of squares and degrees of freedom to the various components.

SINGLE-FACTOR ANOVA

Example



$$SST = SSTR + SSE$$

SINGLE-FACTOR ANOVA

Example

ANOVA table
A table used to summarize the analysis of variance computations and results. It contains columns showing the source of variation, the sum of squares, the degrees of freedom, the mean square, and the *F* value(s).

In Excel use:

- Data → Data Analysis → ANOVA Single Factor

depression

Let's perform for dataset 1: "good health"

SSTR

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	78.53333	2	39.26667	6.773188	0.002296	3.158843
Within Groups	330.45	57	5.797368			
Total	408.9833	59				

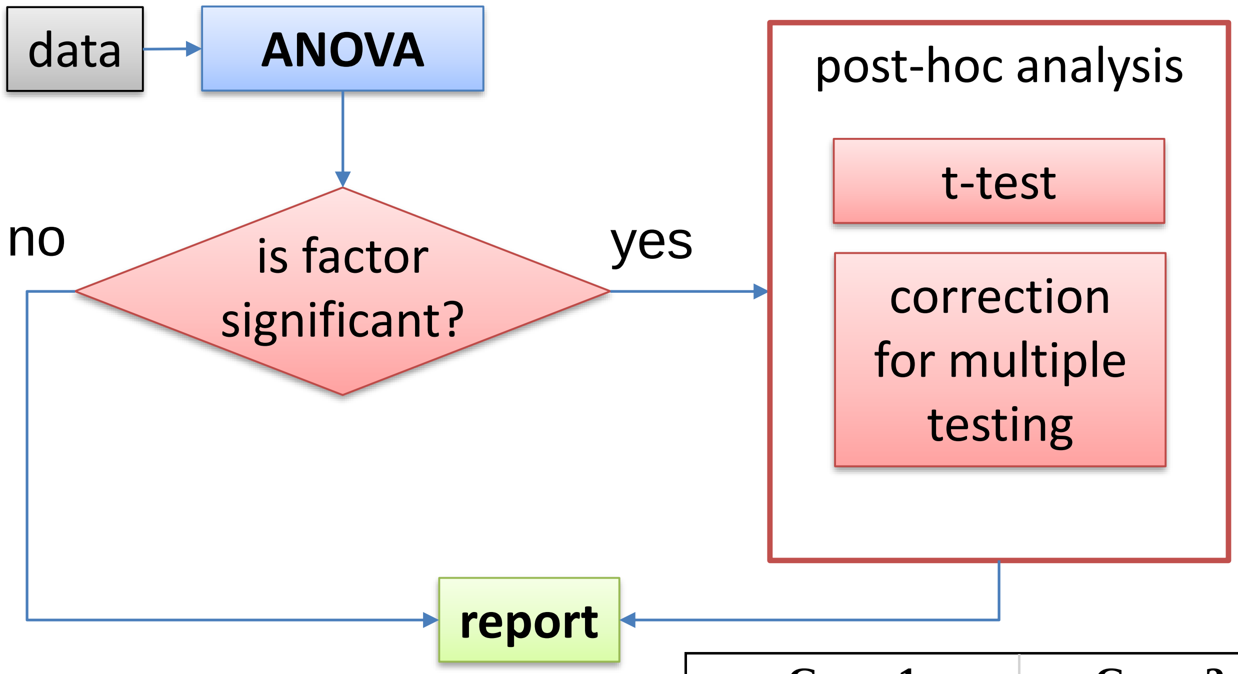
SSE

```
# read dataset
Dep = read.table(
"http://edu.modas.lu/data/
txt/depression2.txt",
  header=T,
  sep="\t",
  as.is=FALSE)
str(Dep)
# consider only healthy
DepGH = Dep[Dep$Health ==
            "good",]
# build 1-way ANOVA model
res1 = aov(Depression ~
           Location, DepGH)
summary(res1)
```

SINGLE-FACTOR ANOVA

Post-hoc Analysis

Post-hoc analysis
allows for additional exploration of significant differences in the data, when significant effect of the factor was already confirmed (for example, by ANOVA).




```r
# build 1-way ANOVA model
res1 = aov(Depression ~
           Location, DepGH)
summary(res1)

# add post-hoc analysis
TukeyHSD(res1)
```

$$FDR = \frac{pval * nComparisons}{k}$$

Group1	Group2	p-value	k	FDR
Florida	New York	0.00021	1	0.00063
Florida	North Carolina	0.0667	2	0.10005
New York	North Carolina	0.11264	3	0.11264

If you can – use **Tukey Honest Significant Differences**

if not – just do FDR-adjustment

MULTI-FACTOR ANOVA

Factors and Treatments

Factor
Another word for the independent variable of interest.

Factorial experiment
An experimental design that allows statistical conclusions about two or more factors.

Treatments
Different levels of a factor.

depression

Factor 1: Health → good health, bad health

Factor 2: Location → Florida, New York, North Carolina

$$\text{Depression} = \mu + \text{Health} + \text{Location} + \text{Health}\times\text{Location} + \varepsilon$$

Interaction
The effect produced when the levels of one factor interact with the levels of another factor in influencing the response variable.

ANOVA example from Partek™

```
# read dataset
Dep = read.table(
"http://edu.modas.lu/data/
txt/depression2.txt",
  header=T,
  sep="\t",
  as.is=FALSE)
str(Dep)
# build 2-way ANOVA model
res2 = aov( Depression ~
  Health + Location+
  Health*Location, Dep)
summary(res2)
# post-hoc
TukeyHSD(res2)
```

MULTI-FACTOR ANOVA

2-factor ANOVA with *r* Replicates

Replications
The number of times each experimental condition is repeated in an experiment.

a = number of levels of factor A
b = number of levels of factor B
r = number of replications
n_T = total number of observations taken in the experiment; $n_T = abr$

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Factor A	SSA	$a - 1$	$\text{MSA} = \frac{\text{SSA}}{a - 1}$	$\frac{\text{MSA}}{\text{MSE}}$
Factor B	SSB	$b - 1$	$\text{MSB} = \frac{\text{SSB}}{b - 1}$	$\frac{\text{MSB}}{\text{MSE}}$
Interaction	SSAB	$(a - 1)(b - 1)$	$\text{MSAB} = \frac{\text{SSAB}}{(a - 1)(b - 1)}$	$\frac{\text{MSAB}}{\text{MSE}}$
Error	SSE	$ab(r - 1)$	$\text{MSE} = \frac{\text{SSE}}{ab(r - 1)}$	
Total	SST	$n_T - 1$		

MULTI-FACTOR ANOVA

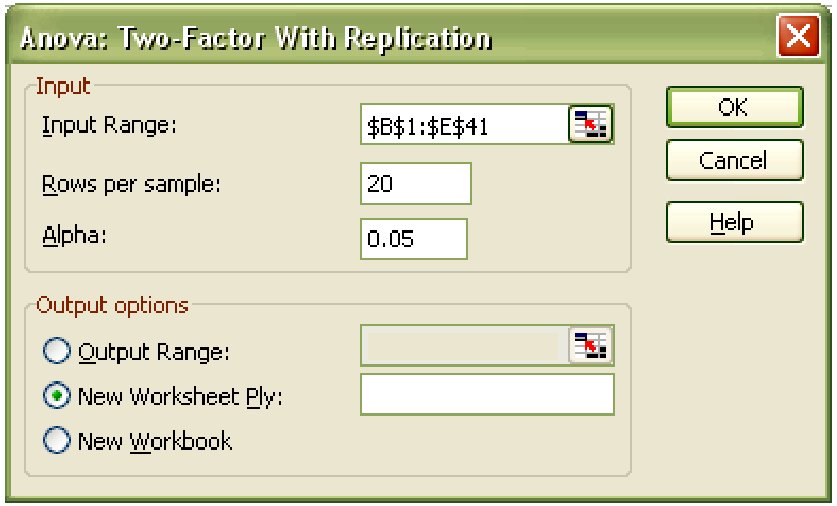
2-factor ANOVA with *r* Replicates: Example

depression.xls → **Factor 1:** Health; **Factor 2:** Location

1. Reorder the data into format understandable for Excel

	Florida	New York	North Carolina
Good health	3	8	10
	7	11	7
	7	9	3
	3	7	5
	…	…	…
	7	7	8
	3	8	11
bad health	**13**	**14**	**10**
	12	**9**	**12**
	17	**15**	**15**
	17	**12**	**18**
	…	…	…
	11	**13**	**13**
	17	**11**	**11**

2. Use Data → Data Analysis → ANOVA: Two-factor with replicates



MULTI-FACTOR ANOVA

Example & Effect size

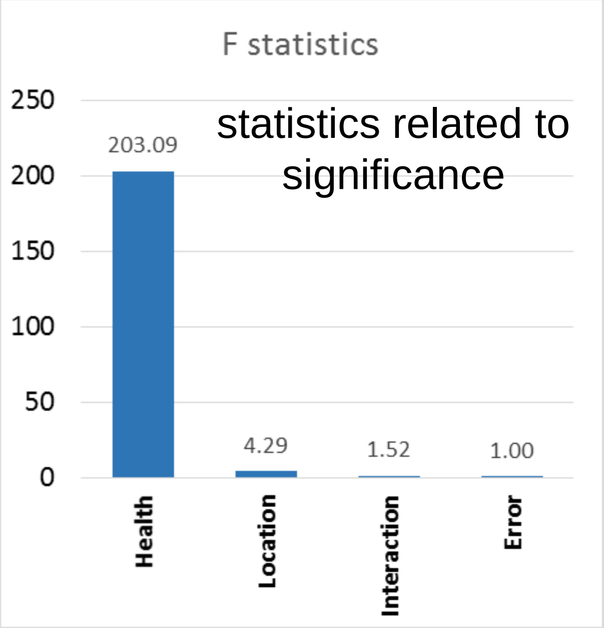
Health
Location
Interaction
Error

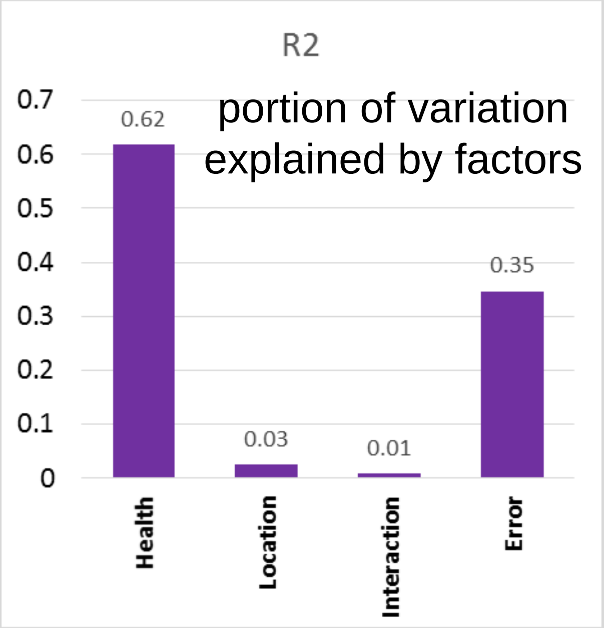
ANOVA

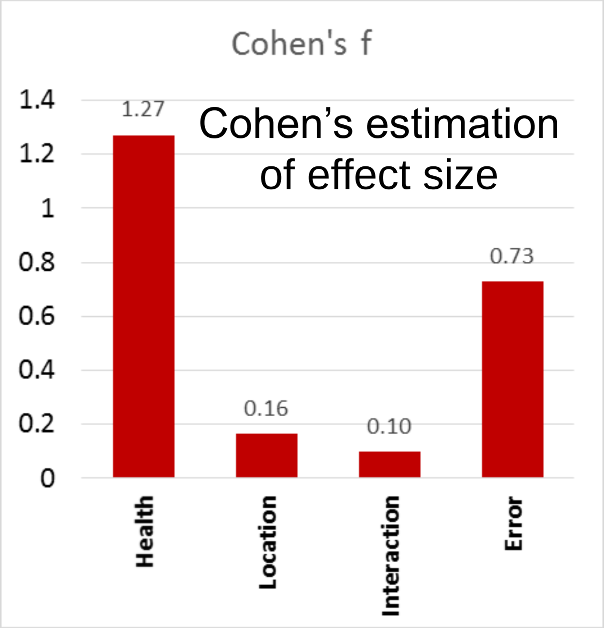
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1748.033	1	1748.033	203.094	4.4E-27	3.92433
Columns	73.85	2	36.925	4.290104	0.015981	3.075853
Interaction	26.11667	2	13.05833	1.517173	0.223726	3.075853
Within	981.2	114	8.607018			
Total	2829.2	119				

η^2 or $R^2 = SSx / SST$

$f = \mathrm{sqrt}(R^2 / (1-R^2))$







MULTI-FACTOR ANOVA

Example 2

salaries.xls

Salary/week	Occupation	Gender
872	Financial Manager	Male
859	Financial Manager	Male
1028	Financial Manager	Male
1117	Financial Manager	Male
1019	Financial Manager	Male
519	Financial Manager	Female
702	Financial Manager	Female
805	Financial Manager	Female
558	Financial Manager	Female
591	Financial Manager	Female

	Ocupation		
Sex	Financial Manage	Computer Programmer	Pharmacist
Male	872	747	1105
	859	766	1144
	1028	901	1085
	1117	690	903
	1019	881	998
Female	519	884	813
	702	765	985
	805	685	1006
	558	700	1034
	591	671	817

Q: Which factors have significant effect on the salary

Data → Data Analysis → ANOVA: Two-factor with replicates

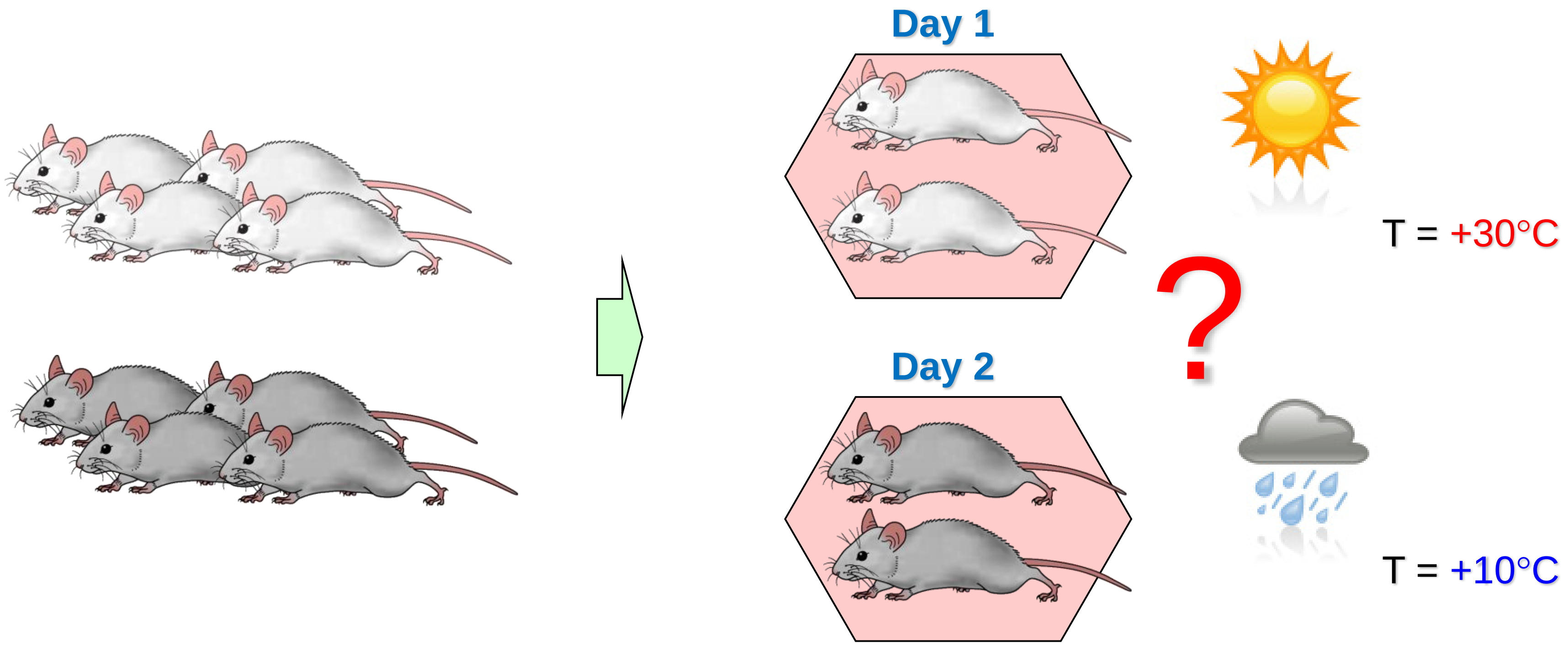
Sourceof Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	221880	1	221880	21.254	0.000112	4.25968
Columns	276560	2	138280	13.246	0.000133	3.40283
Interaction	115440	2	57720	5.5289	0.010595	3.40283
Within	250552	24	10439.7			
Итого	864432	29				

EXPERIMENTAL DESIGN

Experiments

Aware of Batch Effect !

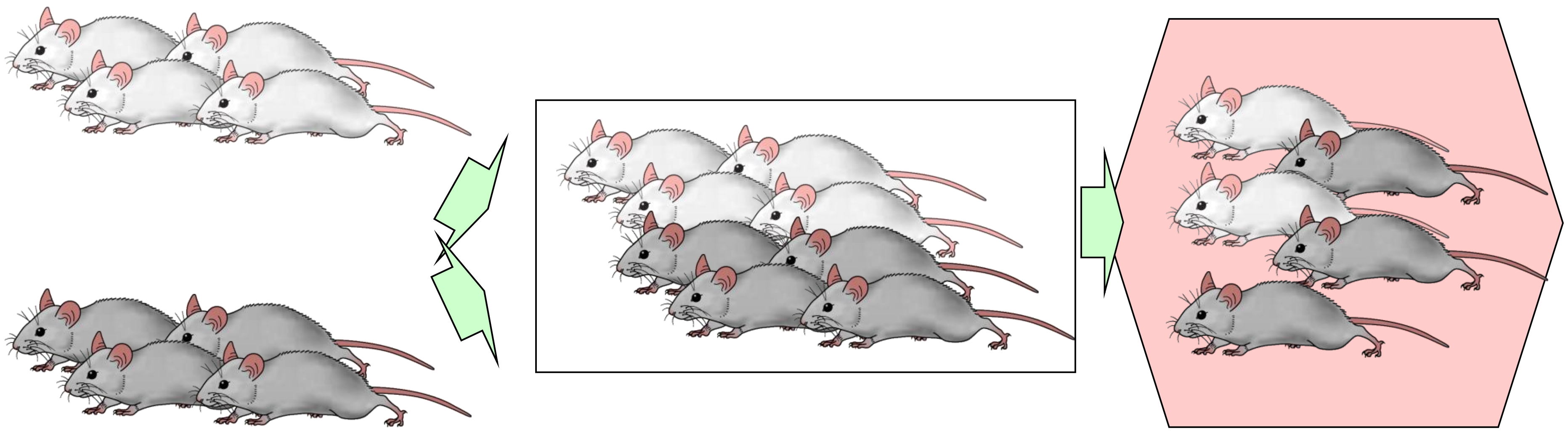
When designing your experiment always remember about various factors which can effect your data: batch effect, personal effect, lab effect...



EXPERIMENTAL DESIGN

Experiments

Completely randomized design
An experimental design in which the treatments are randomly assigned to the experimental units.



We can nicely randomize:

Day effect

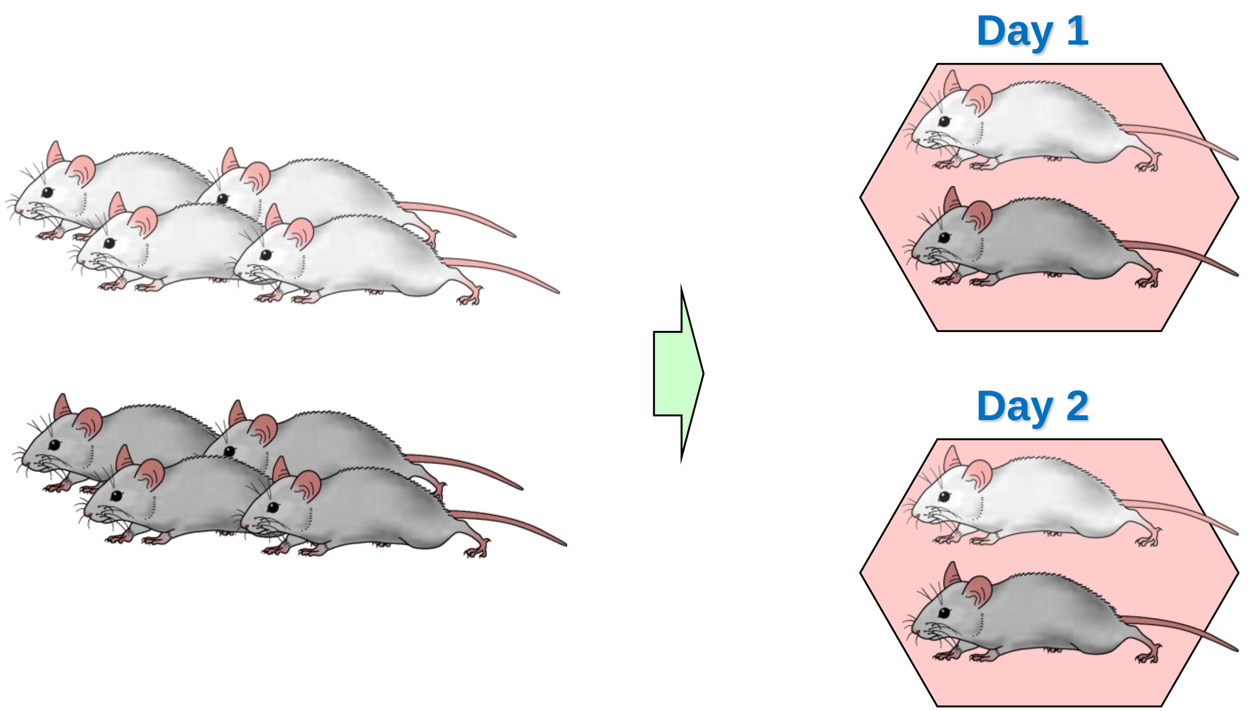
Batch effect

EXPERIMENTAL DESIGN

Experiments

Blocking

The process of using the same or similar experimental units for all treatments. The purpose of blocking is to remove a source of variation from the error term and hence provide a more powerful test for a difference in population or treatment means.



EXPERIMENTAL DESIGN

Experiments

A good suggestion… ☺

Block what you can block, **randomize** what you cannot, and try to **avoid** unnecessary factors

ANOVA

Task

mice.xls

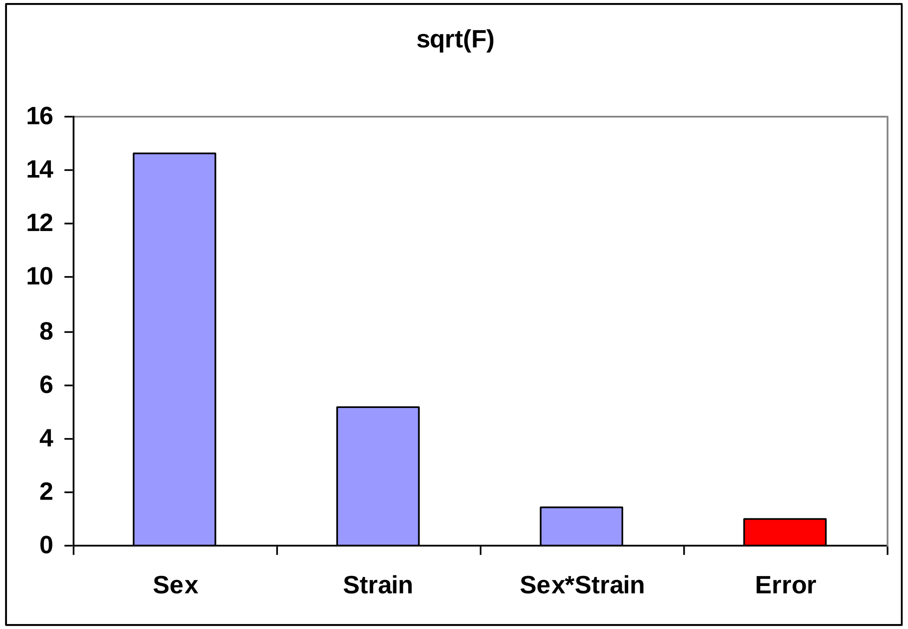
Q: Does mouse strain affect the weight? Show the effects of sex and strain using ANOVA

		129S1/SvImJ	A/J	AKR/J	BALB/cByJ	BTBR_T+_	BUB/BnJ	C3H/HeJ
1	Female	20.5	23.2	24.6	22.8	28	27.1	21.4
2		20.8	22.4	26	23.5	25.8	24.1	28.2
3		19.8	22.7	31	23.8	26	25.9	23.5
4		21	21.4	25.7	22.7	26.5	25.9	23.9
5		21.9	22.6	23.7	19.7	26.3	26	22.8
6		22.1	20	21.1	26.2	27	27.1	18.4
7		21.3	21.8	23.7	24.1	26	26.2	21.8
8		20.1	20.8	24.5	23.5	28.8	27.5	25
9		18.9	19.5	32.3	23.8	28	30.2	20.1
10	Male	24.7	25.8	42.8	29.3	34.1	36.2	31.2
11		27.2	27.7	32.6	32.2	33	36.9	28.2
12		23.9	29.9	34.8	29.7	38.7	34.4	26.7
13		26.3	24.8	32.8	30	39	34.3	29.3
14		26	22.9	34.8	27	31	31.7	33.1
15		23.3	24.5	32.8	30	32	33	28.2
16		26.5	24.6	33.6	33.1	33.7	33.2	31.2
17		27.4	21.6	30.7	30.6	33.1	34	27.7
18		27.5	26.9	36.5	28.7	32.5	31	27.5

ANOVA

Optional Task

mice.xls



Factor	sqrt(F)
Sex	14.64136
Strain	5.193487
Sex*Strain	1.447993
Error	1

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1206.676	1	1206.676	214.3693	3.36E-26	3.940163
Columns	759.13	5	151.826	26.97231	6.06E-17	2.309202
Interaction	59.01074	5	11.80215	2.096684	0.072376	2.309202
Within	540.38	96	5.628958			
Total	2565.197	107				

B.t.w., something is wrong….

Can you find a problem here? ☺

QUESTIONS ?

Thank you for your attention

to be continued…