Experimental Design and Biostatistics (TAMS38)
Lecture 5 – Two-Way ANOVA

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Example 1 - Flight monitoring

A company wants to compare three different control panels for air traffic monitoring.

Each panel was tested for five simulated emergency and two trials were conducted for each combination of the panel and emergency.

The study included 30 trained air traffic controllers who were randomly distributed over the various experiments.

Each experiment measured the time that was spent to take appropriate action.

	Emergency situation				
PANEL	1	2	3	4	5
1	18	31	22	39	15
	16	35	27	36	12
2	13	33	24	35	10
	15	30	21	38	16
3	24	42	40	52	28
	28	46	37	57	24

- How should the data be analyzed?
- What is the conclusion about panels?
- Is the same panel the best for all emergencies?

Two factor model

We consider now an experiment in which results are affected by two factors: A and B with levels $A_1, \ldots, A_a$ and $B_1, \ldots, B_b$, respectively.

For each level combination $A_i B_j$ we do n independent measurements and obtain observations $y_{ij1}, y_{ij2}, \ldots, y_{ijn}$.

Hence, we have in total $a \cdot b \cdot n$ observations:

	B_1	B_2	.	.	.	B_b
A_1	y_{111}	y_{121}	.	.	.	y_{1b1}
	$\vdots$	$\vdots$				$\vdots$
	y_{11n}	y_{12n}	.	.	.	y_{1bn}
A_2	y_{211}	y_{221}	.	.	.	y_{2b1}
	$\vdots$	$\vdots$				$\vdots$
	y_{21n}	y_{22n}	.	.	.	y_{2bn}
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
A_a	y_{a11}	y_{a21}	.	.	.	y_{ab1}
	$\vdots$	$\vdots$				$\vdots$
	y_{a1n}	y_{a2n}	.	.	.	y_{abn}

For each combination A_iB_j we have n observations.

How should we describe the r.v. Y_{ijk} so that we take into account the effect from both factors?

We starts with building up the expected values in an experiment with $a = b = 3$:

	B_1	B_2	B_3
A_1			
A_2			
A_3			

The Additive Model

An **additive model** means that y_{ijk} are observations of

$$Y_{ijk} = \mu + \tau_i + \beta_j + \tilde{\varepsilon}_{ijk},$$
$$(i = 1, \ldots, a,\ j = 1, \ldots, b,\ k = 1, \ldots, n),$$

where $\sum_{i=1}^{a} \tau_i = 0,\ \sum_{j=1}^{b} \beta_j = 0;$ and the r.v. $\tilde{\varepsilon}_{ijk} \sim N(0, \sigma)$ are all independent.

Additivity means that a given A-level have the same effect for all B-levels and that the given B-level have the same effect for all A-levels.

In the additive model we have $1 + (a - 1) + (b - 1)$ free parameters.

A **block effect** is additive and we have usually no interest in block effects itself but we use it to reduce the residual variance.

If some A- and B-levels are especially good, or especially bad together, then we should take that into account by including interaction parameters.

A **complete Two-Way ANOVA** means that y_{ijk} are observations from

$$\begin{aligned} Y_{ijk} &= \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk} \qquad \textbf{(effects model)} \\ &= \mu_{ij} + \varepsilon_{ijk} \qquad \textbf{(means model)} \end{aligned}$$

for $i = 1, \ldots, a,\ j = 1, \ldots, b,\ k = 1, \ldots, n$, where $\sum_{i=1}^{a} \tau_i = 0,\ \sum_{j=1}^{b} \beta_j = 0,\ \sum_{i=1}^{a} (\tau\beta)_{ij} = 0$ for all j, $\sum_{j=1}^{b} (\tau\beta)_{ij} = 0$ for all i and the r.v. $\varepsilon_{ijk} \sim N(0, \sigma)$ are all independent.

- $\tau_1, \ldots, \tau_a$ called main effects A;
- $\beta_1, \ldots, \beta_b$ called main effects B;
- $(\tau\beta)_{ij}$ called interaction effects between A and B.

In the complete model we have

$$1 + (a-1) + (b-1) + (a-1)(b-1) = ab$$

free parameters.

The complete model means that we have a characteristic value μ_{ij} for each combination of levels and no relation between different μ_{ij}.

1. If $n = 1$ we **must** use the additive model.

2. If you can show that the interaction effects are negligible, so we can simplify the model by removing interaction term what leads to the additive model.

3. If the interaction effects are not negligible, we write the model

$$Y_{ijk} = \mu_{ij} + \varepsilon_{ijk}$$

and study effects A_i and B_j through μ_{ij}. We have *abn* samples with the characteristic mean for each of the level combination.

One can do pairwise comparisons between appropriate μ_{ij}, essentially, by utilizing methods for One-Way ANOVA.

Sample means

We have

$$\bar{y}_{i..} = \frac{1}{bn}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk} \qquad \text{(the average value of row } i\text{)}$$

$$\bar{y}_{.j.} = \frac{1}{an}\sum_{i=1}^{a}\sum_{k=1}^{n} y_{ijk} \qquad \text{(the average value of column } j\text{)}$$

$$\bar{y}_{ij.} = \frac{1}{n}\sum_{k=1}^{n} y_{ijk} \qquad \text{(the average value of cell } (i,j)\text{)}$$

$$\bar{y}_{...} = \frac{1}{abn}\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk} \qquad \text{(total average value)}$$

One can show (exercise) that for the complete model we have

- $E(\overline{Y}_{i..}) = \mu + \tau_i$
- $E(\overline{Y}_{.j.}) = \mu + \beta_j$
- $E(\overline{Y}_{ij.}) = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} = \mu_{ij}$
- $E(\overline{Y}_{...}) = \mu$

Point estimators

By estimating the expected value with the average, we get the unbiased estimates

$$\hat{\mu} = \bar{y}_{...}$$
$$\hat{\tau}_i = \bar{y}_{i..} - \bar{y}_{...}$$
$$\hat{\beta}_j = \bar{y}_{.j.} - \bar{y}_{...}$$

and

$$\begin{aligned}\widehat{(\tau\beta)}_{ij} &= \hat{\mu}_{ij} - \hat{\tau}_i - \hat{\beta}_j - \hat{\mu} = \bar{y}_{ij.} - (\bar{y}_{i..} - \bar{y}_{...}) - (\bar{y}_{.j.} - \bar{y}_{...}) - \bar{y}_{...} \\ &= \bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...},\end{aligned}$$

since $\mu_{ij} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij}$.

Analysis of variance (ANOVA)

We can split up the total sum of squares (SS_T) in four new sum of squares, i.e.,

$$
\begin{aligned}
SS_T &= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{...})^2 \\
&= \sum_{i=1}^{a} bn(\bar{y}_{i..} - \bar{y}_{...})^2 + \sum_{j=1}^{b} an(\bar{y}_{.j.} - \bar{y}_{...})^2 \\
&+ \sum_{i=1}^{a}\sum_{j=1}^{b} n(\bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...})^2 + \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{ij.})^2 \\
&= SS_A + SS_B + SS_{AB} + SS_E
\end{aligned}
$$

$$SS_A = \sum_{i=1}^{a} bn(\bar{y}_{i..} - \bar{y}_{...})^2 = \sum_{i=1}^{a} bn\hat{\tau}_i^2$$

$$SS_B = \sum_{j=1}^{b} an(\bar{y}_{.j.} - \bar{y}_{...})^2 = \sum_{j=1}^{b} an\hat{\beta}_j^2$$

$$SS_{AB} = \sum_{i=1}^{a}\sum_{j=1}^{b} n(\bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...})^2 = \sum_{i=1}^{a}\sum_{j=1}^{b} n\widehat{(\tau\beta)}_{ij}^2$$

$$SS_E = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{ij.})^2 = \sum_{i=1}^{a}\sum_{j=1}^{b}(n-1)s_{ij}^2,$$

where s_{ij}^2 is a sample variance of cell (i, j).

We can see that also in this case the sums of squares measure different effects and that SS_E is related to the estimate of σ^2.

ANOVA - Theorem

With the help of Cochran's theorem we can show for the Two-Way ANOVA that

Theorem. Let

$$\begin{aligned} Y_{ijk} &= \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk} \\ &= \mu_{ij} + \varepsilon_{ijk} \end{aligned}$$

where $\sum_{i=1}^{a} \tau_i = 0$, $\sum_{j=1}^{b} \beta_j = 0$, $\sum_{i=1}^{a} (\tau\beta)_{ij} = 0$ for all j, $\sum_{j=1}^{b} (\tau\beta)_{ij} = 0$ for all i and the r.v. $\varepsilon_{ijk} \sim N(0,\ \sigma)$ are all independent.

Then it holds that ($\Rightarrow$ Next slide)

(i) the r.v. SS_A , SS_B , SS_{AB} and SS_E are independent;

(ii) the r.v. $\frac{SS_E}{\sigma^2} \sim \chi^2(ab(n-1))$;

(iii) if $\tau_1 = \ldots = \tau_a = 0$, then $\frac{SS_A}{\sigma^2} \sim \chi^2(a-1)$;

(iv) if $\beta_1 = \ldots = \beta_b = 0$, then $\frac{SS_B}{\sigma^2} \sim \chi^2(b-1)$;

(v) if $(\tau\beta)_{ij} = 0$ for all (i, j), then

$$\frac{SS_{AB}}{\sigma^2} \sim \chi^2((a-1)(b-1)).$$

One can also show that the r.v. $\overline{Y}_{i..}$, $\overline{Y}_{.j.}$, $\overline{Y}_{ij.}$ and $\overline{Y}_{...}$ are independent of the r.v. SS_E . This is important in the construction of confidence intervals.

From the theorem follows:

1. σ^2 is estimated with $s^2 = \frac{SS_E}{ab(n-1)}$ with $ab(n-1)$ degrees of freedom.
2. $H_{0A}: \; \tau_1 = \ldots = \tau_a = 0$, i.e., there is no effect of factor A against H_{1A}: not all τ_i equals 0, is tested on level α using the test statistic

$$v_A = \frac{SS_A/(a-1)}{SS_E/[ab(n-1)]} = \frac{SS_A/(a-1)}{s^2}.$$

   H_{0A} is rejected if $v_A > c$ where c is given in $F(a-1, ab(n-1))$-table.
3. $H_{0B}: \; \beta_1 = \ldots = \beta_b = 0$ is tested in the same way.

4. $H_{0AB}: \ (\tau\beta)_{ij} = 0$ for $i = 1, \ldots, a, \ j = 1, \ldots, b$ against H_{1AB} : not all ; $(\tau\beta)_{ij}$ equals zero, is tested on level α with use of the test statistic

$$v_{AB} = \frac{SS_{AB}/[(a-1)(b-1)]}{SS_E/[ab(n-1)]}.$$

H_{0AB} is rejected if $v_{AB} > c'$ where c' is given $F[(a-1)(b-1), ab(n-1)]$-table.

Remark. For 1-4 the degrees of freedom for SS_E comes from the assumption that we use the complete Two-Way ANOVA.

Example 2 - The growth rates of cracks

In an experiment cracks in a material have been studied. Test pieces of the material have been subject loaded in cycles of three different frequencies in three different environments. The growth rate of fatigue cracks have been measured. Results:

	Environments		
Frequency	Air	Water	Salty water
10	2.29	2.06	1.90
	2.47	2.05	1.93
	2.48	2.23	1.75
	2.12	2.03	2.06
1	2.65	3.20	3.10
	2.68	3.18	3.24
	2.06	3.96	3.98
	2.38	3.64	3.24
0.1	2.24	11.00	9.96
	2.71	11.00	10.01
	2.81	9.06	9.36
	2.08	11.30	10.40

We put data in column c1.

```
MTB > set c2
DATA> (1,2,3)4
DATA> (1,2,3)4
DATA> (1,2,3)4
DATA> end
MTB > set c3
DATA> (1,2,3)12
DATA> end
MTB > name c1 'Y' c2 'F' c3 'E'
MTB > table c2 c3;
SUBC> means c1.
```

Tabulated statistics: F, E

```
Rows: F   Columns: E

          1       2      3    All
1     2.340   2.092  1.910  2.114
2     2.442   3.495  3.390  3.109
3     2.460  10.590  9.932  7.661
All   2.414   5.393  5.078  4.295

Cell Contents:  Y  :  Mean
```

We want to analyze data according to the model:

with the following Minitab-analysis.

```
MTB > ANOVA 'Y' = F| E;
SUBC>   GNormalplot;
SUBC>   GFits;
SUBC>   NoDGraphs;
SUBC>   GVars 'F' 'E'.
```

ANOVA: Y versus F, E

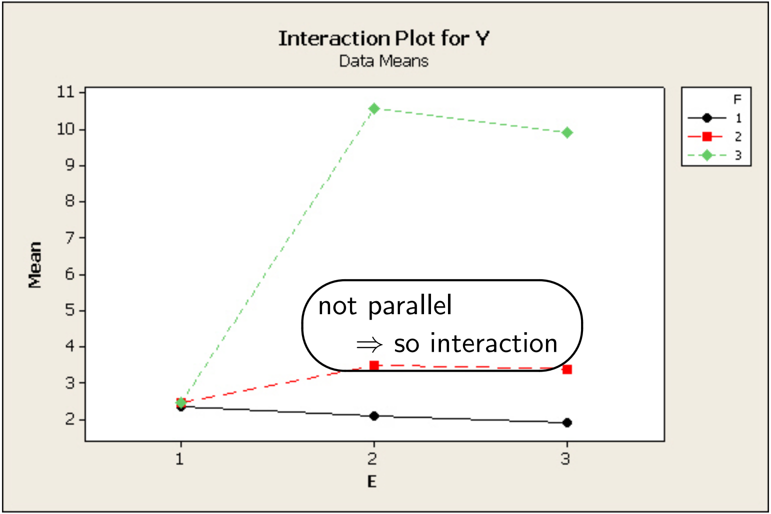
Factor	Type	Levels	Values
F	fixed	3	1, 2, 3
E	fixed	3	1, 2, 3

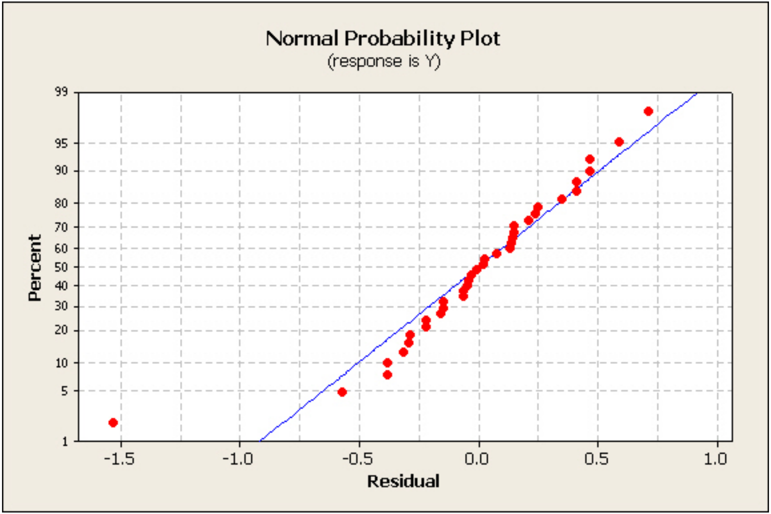
Analysis of Variance for Y

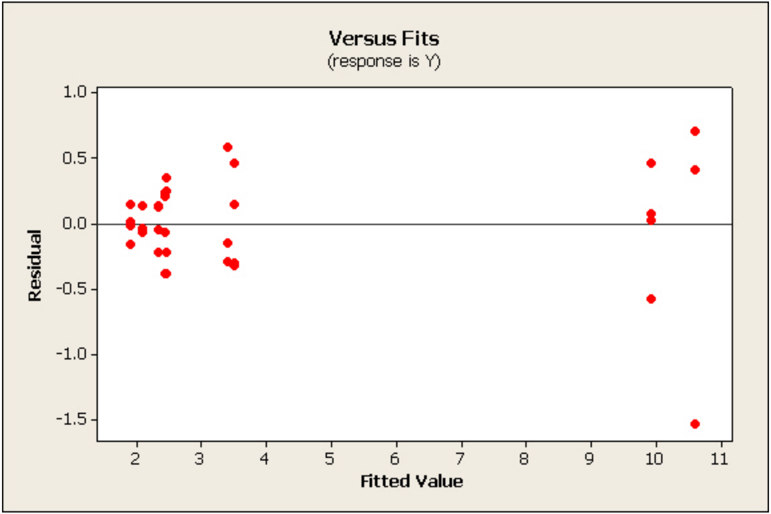
Source	DF	SS	MS	F	P
F	2	209.893	104.946	522.40	0.000
E	2	64.252	32.126	159.92	0.000
F*E	4	101.966	25.491	126.89	0.000
Error	27	5.424	0.201		
Total	35	381.535			

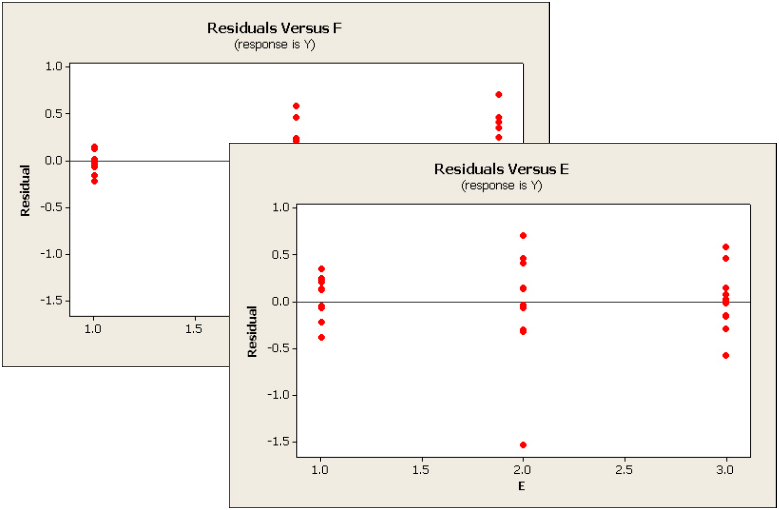
S = 0.448211   R-Sq = 98.58%   R-Sq(adj) = 98.16%

Test the significance of the interaction factor.


Interaction Plot for Y
Data Means
Mean
11
10
9
8
7
6
5
4
3
2
1
2
3
E
F
1
2
3
not parallel
⇒ so interaction


Normal Probability Plot
(response is Y)
Percent
99
95
90
80
70
60
50
40
30
20
10
5
1
-1.5
-1.0
-0.5
0.0
0.5
1.0
Residual


Versus Fits
(response is Y)
1.0
0.5
0.0
-0.5
-1.0
-1.5
Residual
2
3
4
5
6
7
8
9
10
11
Fitted Value


Residuals Versus F
(response is Y)
Residual
1.0
0.5
0.0
-0.5
-1.0
-1.5
1.0
1.5
Residuals Versus E
(response is Y)
Residual
1.0
0.5
0.0
-0.5
-1.0
-1.5
1.0
1.5
2.0
2.5
3.0
E

Additive model

So far, we have studied the complete Two-Way ANOVA. If we have reason to believe that the factors A and B are additive, we can instead work with the additive model.

Sum of squares become different and we have

$$SS_E' = SS_E + SS_{AB} = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}\left(y_{ijk} - \hat{\mu} - \hat{\tau}_i - \hat{\beta}_j\right)^2$$
$$= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...})^2,$$

so we have the estimated expectation

$$\widehat{E(Y_{ijk})} = \hat{\mu} + \hat{\tau}_i + \hat{\beta}_j = \bar{y}_{i..} + \bar{y}_{.j.} - \bar{y}_{...}.$$

Theorem. Let

$$Y_{ijk} = \mu + \tau_i + \beta_j + \tilde{\varepsilon}_{ijk},$$

where $\sum_{i=1}^{a} \tau_i = 0$, $\sum_{j=1}^{b} \beta_j = 0$, and the r.v. $\tilde{\varepsilon}_{ijk}$ are independent and $N(0, \tilde{\sigma})$. Then it holds that

(i) the r.v. SS_A , SS_B and SS_E' are independent;

(ii) the r.v. $\dfrac{SS_E'}{\tilde{\sigma}^2} \sim \chi^2(ab(n-1) + (a-1(b-1)) =$
$= \chi^2(abn - a - b + 1)$;

(iii) if $\tau_1 = \ldots = \tau_a = 0$, then $\dfrac{SS_A}{\tilde{\sigma}^2} \sim \chi^2(a-1)$;

(iv) if $\beta_1 = \ldots = \beta_b = 0$, then $\dfrac{SS_B}{\tilde{\sigma}^2} \sim \chi^2(b-1)$;

Consequences:

1. $\tilde{\sigma}^2$ is estimated with

$$\tilde{s}^2 = \frac{SS'_E}{abn - a - b + 1},$$

with degrees of freedom df$= abn - a - b + 1$.

2. $H_{0A} : \ldots$ is tested with use of $v_A = \ldots$ etc.

Pairwise comparisons for the main effects

When using the additive model, we compare the levels of a factor by comparing main effects.

$$\hat{\tau}_i - \hat{\tau}_\nu = \left(\overline{Y}_{i..} - \overline{Y}_{...}\right) - \left(\overline{Y}_{\nu..} - \overline{Y}_{...}\right)$$
$$= \overline{Y}_{i..} - \overline{Y}_{\nu..} \sim N\left(\tau_i - \tau_\nu, \sqrt{\frac{2\tilde{\sigma}^2}{bn}}\right)$$

- t-interval with Bonferroni estimation of the confidence level, or
- Tukey methods.

Important to keep track of the degrees of freedom for $\tilde{\sigma}^2$-estimates.

Example 1, cont (Flight monitoring)

Analyzing the complete model gives

```
MTB > ANOVA 'Y' = P|NS.
```

ANOVA: Y versus P, NS

Factor	Type	Levels	Values
P	fixed	3	1, 2, 3
NS	fixed	5	1, 2, 3, 4, 5

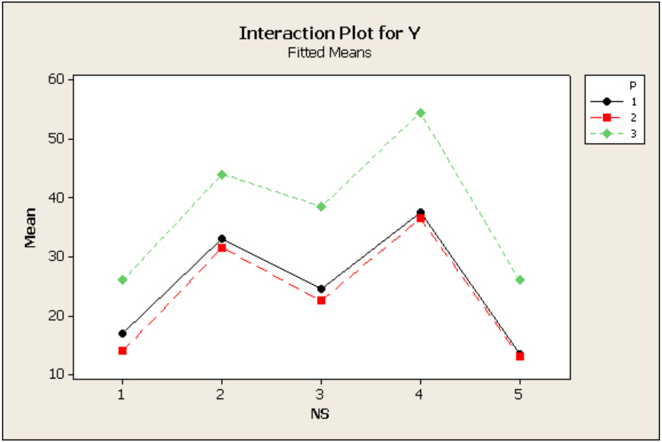
Analysis of Variance for Y

Source	DF	SS	MS	F	P
P	2	1227.80	613.90	86.87	0.000
NS	4	2850.13	712.53	100.83	0.000
P*NS	8	44.87	5.61	0.79	0.617
Error	15	106.00	7.07		
Total	29	4228.80			

S = 2.65832   R-Sq = 97.49%   R-Sq(adj) = 95.15%

Using Minitab we conclude that we can use the additive model (as $P_{\text{samspel}} = 0.617$), i.e.,

$$Y_{ijk} = \mu + \tau_i + \beta_j + \tilde{\varepsilon}_{ijk}.$$



Analyze the additive model.

```
MTB > ANOVA 'Y' = P NS.
```

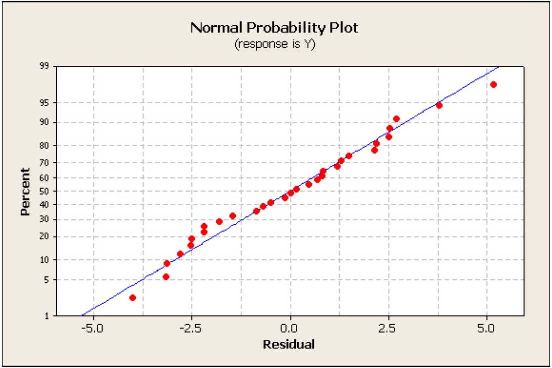
ANOVA: Y versus P, NS

```
Factor  Type   Levels  Values
P       fixed       3  1, 2, 3
NS      fixed       5  1, 2, 3, 4, 5

Analysis of Variance for Y

Source  DF       SS      MS       F      P
P        2  1227.80  613.90   93.59  0.000
NS       4  2850.13  712.53  108.63  0.000
Error   23   150.87    6.56
Total   29  4228.80

S = 2.56114   R-Sq = 96.43%   R-Sq(adj) = 95.50%
```

Means

P	N	Y
1	10	25.100
2	10	23.500
3	10	37.800

NS	N	Y
1	6	19.000
2	6	36.167
3	6	28.500
4	6	42.833
5	6	17.500



We construct the confidence interval for the differences $\tau_i - \tau_\nu$ using Tukeys method. We obtain

$$\hat{\tau}_i - \hat{\tau}_\nu = (\bar{y}_{i..} - \bar{y}_{...}) - (\bar{y}_{\nu..} - \bar{y}_{...}) = \bar{y}_{i..} - \bar{y}_{\nu..}$$

Then, the r.v. $\overline{Y}_{i..} \sim N\left(\mu + \tau_i,\ \frac{\tilde{\sigma}}{\sqrt{10}}\right)$ and $\tilde{s}^2 = \dfrac{SS_E}{23} = 6.559$ with degrees of freedom df$= 23$. Hence, we have interval

$$\begin{aligned} I_{\tau_i - \tau_\nu} &= \left(\bar{y}_{i..} - \bar{y}_{\nu..} \mp q_{0.05}(3,23)\ \frac{\tilde{s}}{\sqrt{10}}\right) \\ &= \left(\bar{y}_{i..} - \bar{y}_{\nu..} \mp 3.54\ \frac{2.561}{\sqrt{10}}\right) = (\bar{y}_{i..} - \bar{y}_{\nu..} \mp 2.867) \end{aligned}$$

with $\bar{y}_{1..} = 25.1$; $\bar{y}_{2..} = 23.5$; $\bar{y}_{3..} = 37.8$.

We see that panel 3 is significantly slower than panel 1 and 2. No significant differences can be seen between panel 1 and 2.

Discussion of the methods

The models we have discussed are based on assumptions about **normal distribution** and **constant variance**.

Such a model is of course an approximation of real life.

Sometimes you can achieve better adjustment to the normal distribution and more stable variance by transforming data, see e.g., hand in assignment 1.

When using a Gaussian random variable to describe a positive measurement value, then the probability mass of the negative axis should be negligible, i.e., it is desirable to

$$\mu - 3\sigma > 0.$$

Non-parametric method

We will now use non-parametric methods on a design with blocking factor.

Suppose we have a experiment where we used t different treatments precisely one time withing each of the b blocks, where each block have t equivalent experimental units.

We want to test

$$H_0 : \text{No difference between treatments.}$$

vs.

$$H_1 : \text{There is difference between treatments.}$$

Let y_{ij} denote measurement for treatment i within block j.

Friedmans test

Procedure:

1. Rank $(1, \ldots, t)$ the observations within each block and let

$$s_i = \text{ sum of ranks for treatment } i.$$

2. Calculate test statistic

$$T = \begin{cases} \dfrac{12S_t}{t(t+1)} - 3b(t+1), & \text{(no ties)}, \\[2ex] \dfrac{b(t-1)(S_t - C)}{S_r - C}, & \text{(ties)}, \end{cases}$$

where

$$S_r = \sum_{i,j} r_{ij}^2, \quad S_t = \frac{1}{b}\sum_i s_i^2, \quad C = \frac{1}{4}bt(t+1)^2.$$

3. H_0 is rejected if $T \geq c$, where c is calculated from the condition

$$\alpha = P(T \geq c \text{ given } H_0 \text{ is true}).$$

(i) For small b and t we use Table to find c.

(ii) For large values of b and t we use the fact that T is *appr* $\chi^2(t-1)$ to obtain c.

Explanation of the test statistic: Test statistic can be writen as

$$T = \frac{12b}{t(t+1)} \sum_{i=1}^{t} \left[\frac{s_i}{b} - \frac{1}{2}(t+1) \right]^2$$

which shows that for each treatment it compares average rank $\frac{s_i}{b}$ with the average of ranks $1, 2, \ldots, t$.

Example 3 (Lehmann, 1975)

Suppose we have three sedatives that we want to test. We have twelve people to try out and we divide those twelve people in four different patient groups, patients in each group is expected to react quite similar to the sedative.

Treatment/Group	1	2	3	4
1	11.0	10.2	11.1	11.2
2	10.1	10.8	9.7	10.4
3	9.8	9.9	10.3	10.9

After we have observed reactions of those various patients to the sedative we ranked the measurements within each block (of patients) and get the following table

Treatment/Group	1	2	3	4	s_i
1	3	2	3	3	11
2	2	3	1	1	7
3	1	1	2	2	6

Then, we have

$$\begin{aligned} t &= 3 \text{ treatments}, \quad (s = 3), \\ b &= 4 \text{ blocks}, \quad (N = 4), \\ s_1 &= 11,\ s_2 = 7,\ s_3 = 6 \end{aligned}$$

and test statistic (no ties)

$$T = \frac{12}{4 \cdot 3 \cdot 4}(11^2 + 7^2 + 6^2) - 3 \cdot 4 \cdot 4 = 3.50.$$

Significance level $\alpha \leq 0.10$ gives $c = 6.0$ with $\alpha = 0.069$.

$\Rightarrow$ $3.5 < 6.0$ so there is not any significant difference between the sedatives.

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