9. Non-parametric statistics - Kruskal Wallis

Lieven Clement

statOmics, Ghent University (https://statomics.github.io)

Contents

1 Comparison of g groups **1**

2 DMH example **1**
2.1 Comet Assay: 1

3 Kruskal-Wallis Rank Test **8**
3.1 DNA Damage Example 9

1 Comparison of g groups

- Extend F-test from a one-way ANOVA to non-parametric alternatives.

2 DMH example

Assess genotoxicity of 1,2-dimethylhydrazine dihydrochloride (DMH) (EU directive)

- 24 rats
- four groups with daily DMH dose
  - control
  - low
  - medium
  - high
- Genotoxicity in liver using comet assay on 150 liver cells per rat.
- Are there differences in DNA damage due to DMH dose?

2.1 Comet Assay:

- Visualise DNA strand breaks
- Length comet tail is a proxy for strand breaks.

```
dna <- read_delim("https://raw.githubusercontent.com/GTPB/PSLS20/master/data/dna.txt", delim = " ")
dna$dose <- as.factor(dna$dose)
dna
```

```
# A tibble: 24 x 3
   id    length dose
   <chr>  <dbl> <fct>
 1 Rat1    19.3 0
 2 Rat2    18.9 0
 3 Rat3    18.6 0
 4 Rat4    19.0 0
```

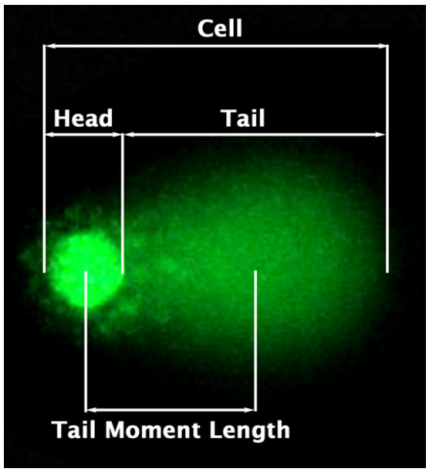
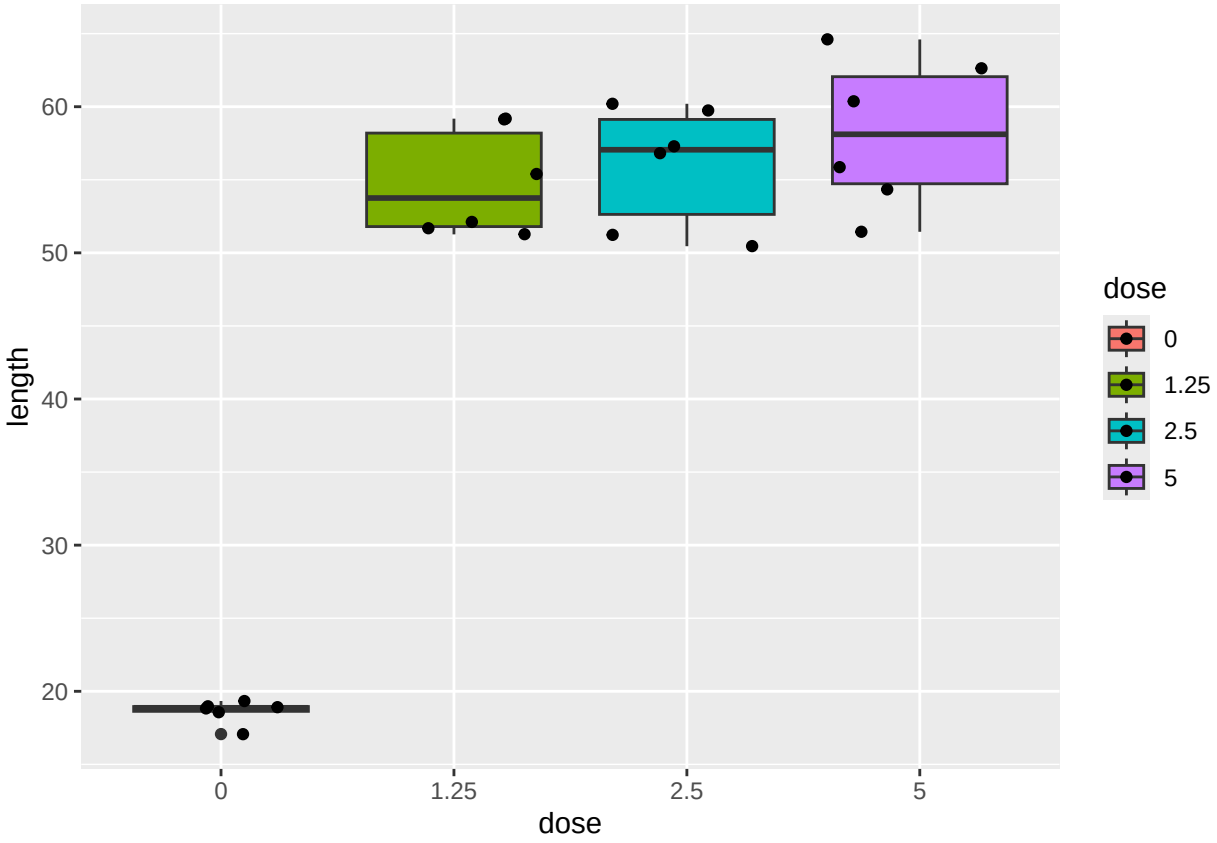


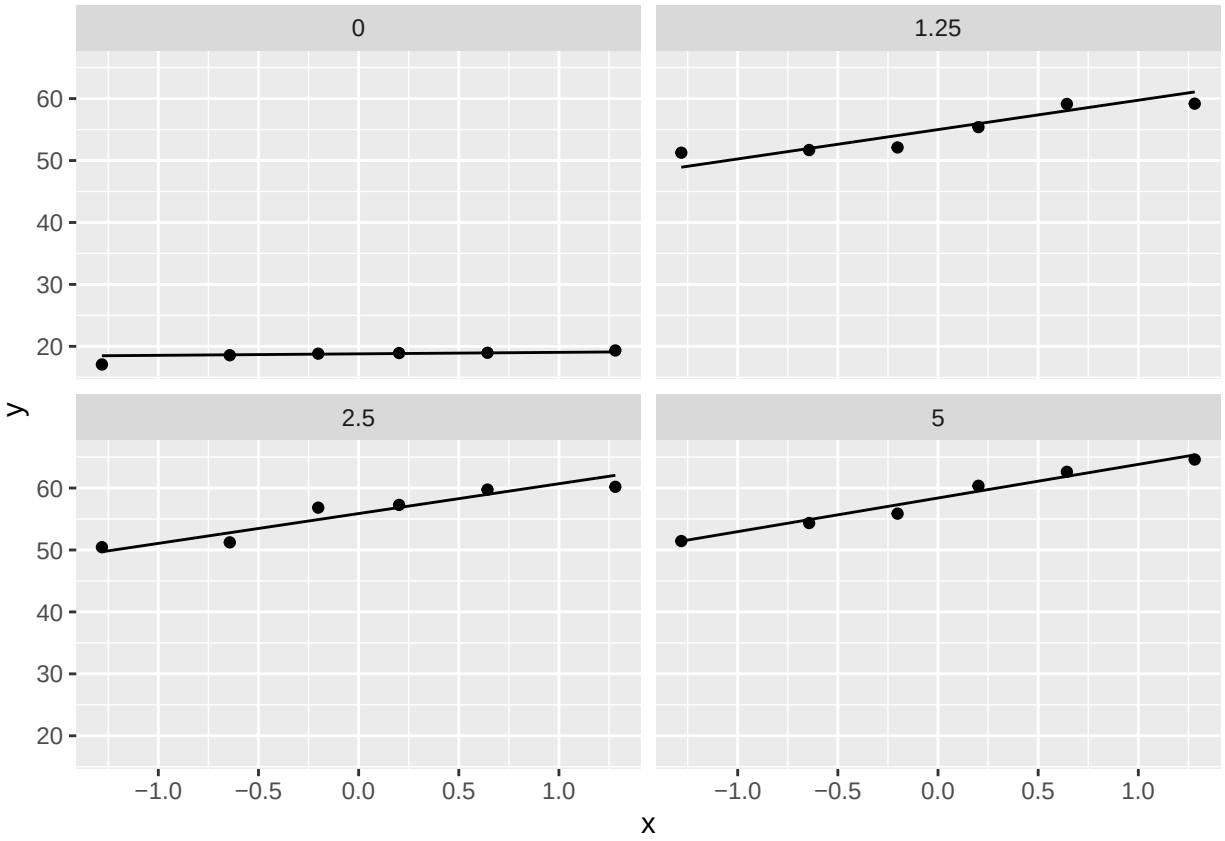
Figure 1: Comet assay

```
 5 Rat5    17.1 0
 6 Rat6    18.8 0
 7 Rat7    55.4 1.25
 8 Rat8    59.2 1.25
 9 Rat9    59.1 1.25
10 Rat10   52.1 1.25
# i 14 more rows
```

```
dna %>%
  ggplot(aes(x = dose, y = length, fill = dose)) +
  geom_boxplot() +
  geom_point(position = "jitter")
```

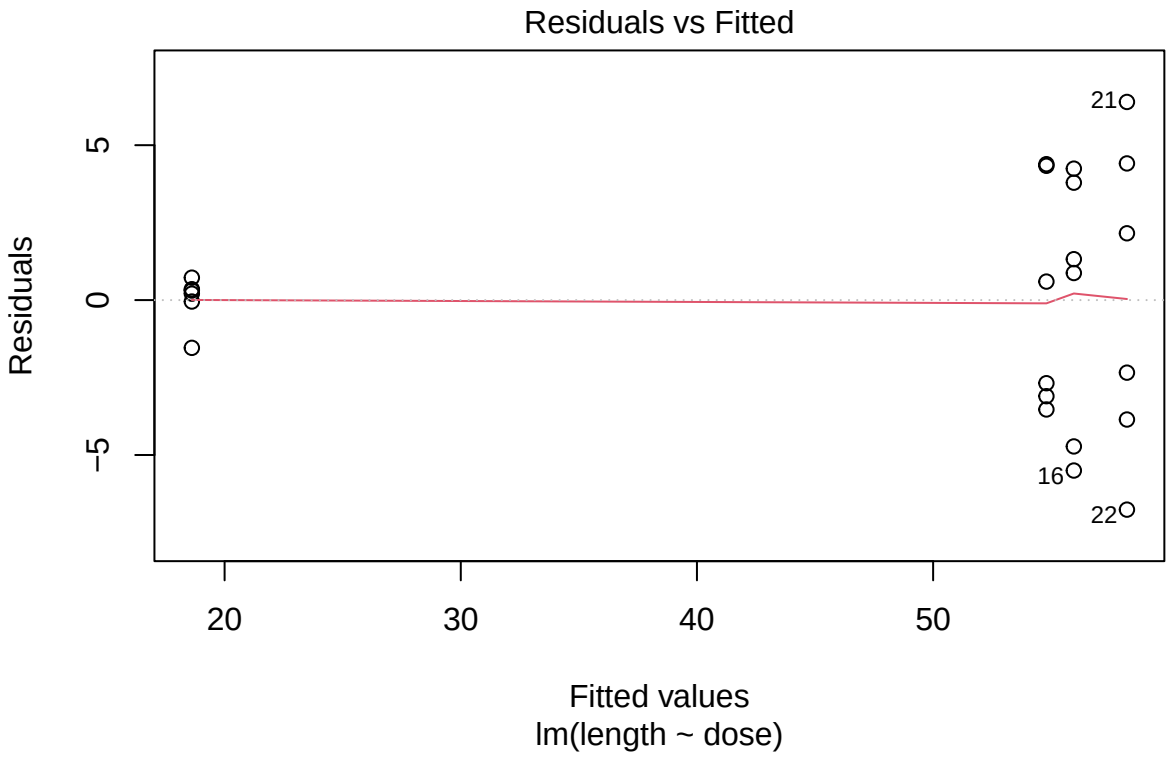


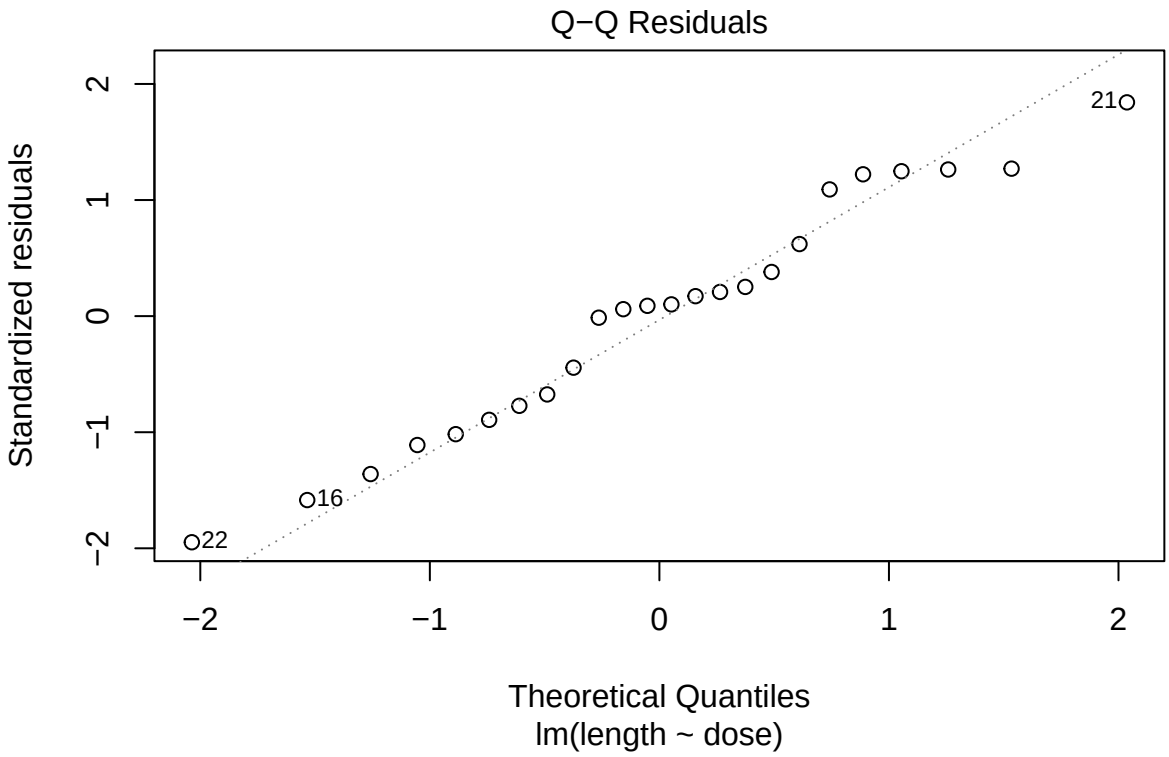

```
dna %>%
  ggplot(aes(sample = length)) +
  geom_qq() +
  geom_qq_line() +
  facet_wrap(~dose)
```

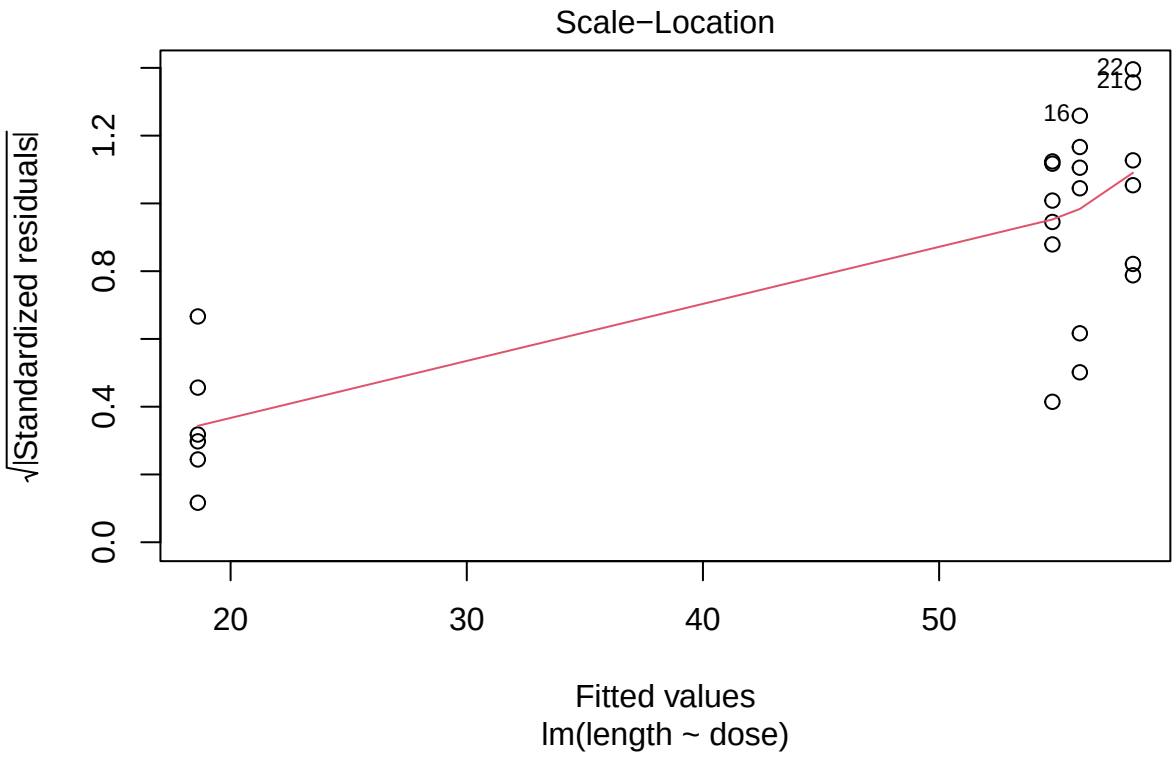


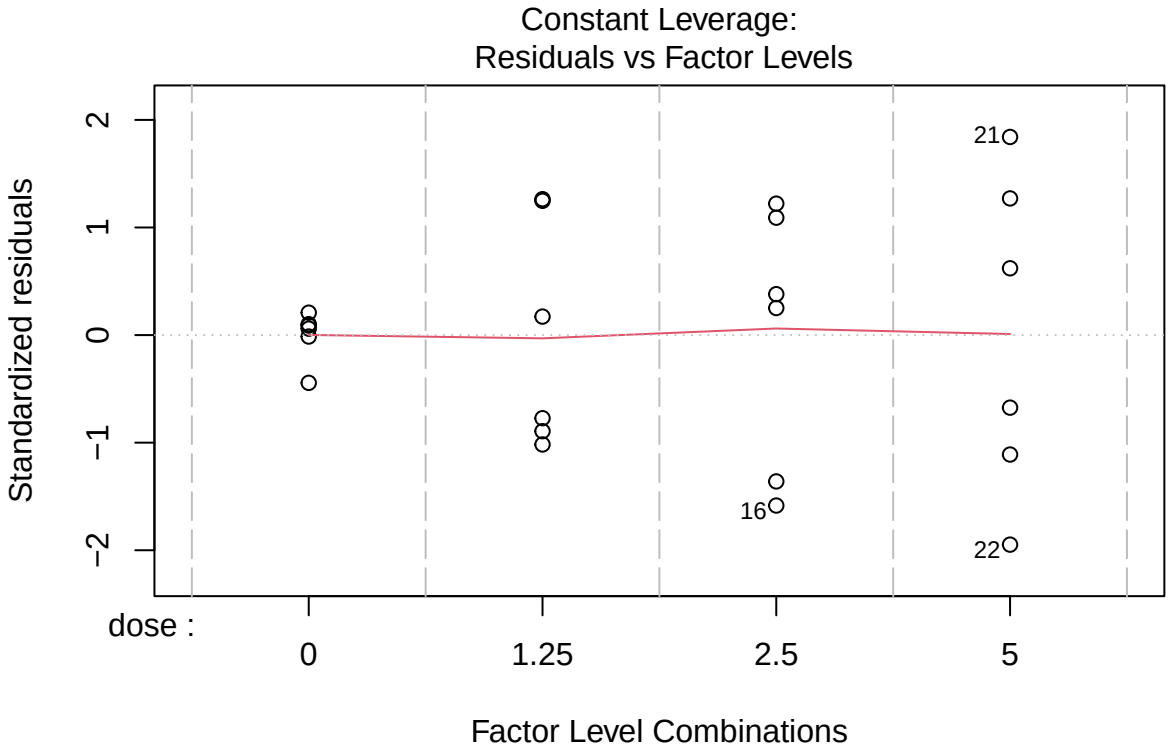
- Strong indication that data in control group has a lower variance.
- 6 observations per group are too few to check the assumptions

```
plot(lm(length ~ dose, data = dna))
```


Residuals vs Fitted
Residuals
5
0
−5
21
16
22
20
30
40
50
Fitted values
lm(length ~ dose)


Q−Q Residuals
Standardized residuals
−2
−1
0
1
2
22
16
21
−2
−1
0
1
2
Theoretical Quantiles
lm(length ~ dose)


Scale−Location
√|Standardized residuals|
0.0
0.4
0.8
1.2
20
30
40
50
Fitted values
lm(length ~ dose)
22
21
16
8



3 Kruskal-Wallis Rank Test

- The Kruskal-Wallis Rank Test (KW-test) is a non-parameteric alternative for ANOVA F-test.
- Classical F-teststatistic can be written as

$$F = \frac{\text{SST}/(g-1)}{\text{SSE}/(n-g)} = \frac{\text{SST}/(g-1)}{(\text{SSTot} - \text{SST})/(n-g)},$$

- with g the number of groups.
- SSTot depends only on outcomes $\mathbf{y}$ and will not vary in permutation test.
- SST can be used as statistic

$$\text{SST} = \sum_{j=1}^{t} n_j (\bar{Y}_j - \bar{Y})^2$$

- The KW test statistic is based on SST on rank-transformed outcomes[1],

$$\text{SST} = \sum_{j=1}^{g} n_j \left(\bar{R}_j - \bar{R}\right)^2 = \sum_{j=1}^{t} n_j \left(\bar{R}_j - \frac{n+1}{2}\right)^2,$$

- with $\bar{R}_j$ the mean of the ranks in group j, and $\bar{R}$ the mean of all ranks,

$$\bar{R} = \frac{1}{n}(1 + 2 + \cdots + n) = \frac{1}{n}\frac{1}{2}n(n+1) = \frac{n+1}{2}.$$

[1] we assume that no *ties* are available

- The KW teststatistic is given by

$$KW = \frac{12}{n(n+1)} \sum_{j=1}^{g} n_j \left(\bar{R}_j - \frac{n+1}{2} \right)^2 .$$

- The factor $\frac{12}{n(n+1)}$ is used so that KW has a simple asymptotic null distribution. In particular under H_0, given thart $\min(n_1, \ldots, n_g) \to \infty$,

$$KW \to \chi^2_{t-1}.$$

- The exact KW-test can be executed by calculating the permutation null distribution (that only depends on $n_1, \ldots, n_g$) to test

$$H_0 : f_1 = \ldots = f_g \text{ vs } H_1 : \text{ at least two means are different.}$$

- In order to allow H_1 to be formulated in terms of means, the assumption of locations shift should be valid.
- For DMH example this is not the case.
- If location-shift is invalid, we have to formulate H_1 in terms of probabilistic indices:

$$H_0 : f_1 = \ldots = f_g \text{ vs } H_1 : \exists\ j, k \in \{1, \ldots, g\} : \mathrm{P}\left[Y_j \geq Y_k\right] \neq 0.5$$

3.1 DNA Damage Example

```
kruskal.test(length ~ dose, data = dna)
```

```
    Kruskal-Wallis rank sum test

data:  length by dose
Kruskal-Wallis chi-squared = 14, df = 3, p-value = 0.002905
```

- On the 5% level of significance we can reject the null hypothesis.
- R-functie `kruskal.test` only returns the asymptotic approximation for p-values.
- With only 6 observaties per groep, this is not a good approximation of the p-value
- With the `coin` R package we can calculate the exacte p-value

```
library(coin)
kwPerm <- kruskal_test(length ~ dose,
  data = dna,
  distribution = approximate(B = 100000)
)
kwPerm
```

```
    Approximative Kruskal-Wallis Test

data:  length by dose (0, 1.25, 2.5, 5)
chi-squared = 14, p-value = 0.00043
```

- We conclude that the difference in the distribution of the DNA damages due to the DMH dose is extremely significantly different.
- Posthoc analysis with WMW tests.

```r
pairwise.wilcox.test(dna$length, dna$dose)
```

```
    Pairwise comparisons using Wilcoxon rank sum exact test

data:  dna$length and dna$dose

     0     1.25  2.5
1.25 0.013 -     -
2.5  0.013 0.818 -
5    0.013 0.721 0.788

P value adjustment method: holm
```

- All DMH behandelingen are significantly different from the control.
- The DMH are not significantly different from one another.
- U1 does not occur in the `pairwise.wilcox.test` output. Point estimate on probability on higher DNA-damage?

```r
nGroup <- table(dna$dose)
probInd <- combn(levels(dna$dose), 2, function(x) {
  test <- wilcox.test(length ~ dose, subset(dna, dose %in% x))
  return(test$statistic / prod(nGroup[x]))
})
names(probInd) <- combn(levels(dna$dose), 2, paste, collapse = "vs")
probInd
```

```
  0vs1.25    0vs2.5      0vs5 1.25vs2.5   1.25vs5    2.5vs5
0.0000000 0.0000000 0.0000000 0.4444444 0.2777778 0.3333333
```

Because there are doubts on the location-shift model we draw our conclusions in terms of the probabilistic index.

3.1.1 Conclusion

- There is an extremely significant difference in in the distribution of the DNA-damage measurements due to the treatment with DMH ($p < 0.001$ KW-test).
- DNA-damage is more likely upon DMH treatment than in the control treatment (all p=0.013, WMW-testen).
- The probability on higher DNA-damage upon exposure to DMH is 100% (Calculation of a CI on the probabilistic index is beyond the scope of the course)
- There are no significant differences in the distributions of the comit-lengths among the treatment with the different DMH concentrations ($p = 0.72$-0.82).
- DMH shows already genotoxic effects at low dose.
- (Alle paarswise tests are gecorrected for multiple testing using Holm's methode).