When we carry out an ANOVA on k treatments, we test

\[ H_0 : \mu_1 = \cdots = \mu_k \quad \text{versus} \quad H_a : H_0 \text{ is false} \]

Assume we reject the null hypothesis, i.e. we have some evidence that not all treatment means are equal. Then we could for example be interested in which ones are the same, and which ones differ. For this, we might have to carry out some more hypothesis tests.

\[ \rightarrow \] Multiple comparisons.
Types of multiple comparisons

There are two different types of multiple comparisons procedures:

Sometimes we already know in advance what questions we want to answer. Those comparisons are called planned (or a priori) comparisons.

Sometimes we do not know in advance what questions we want to answer, and the judgement about which group means will be studied the same depends on the ANOVA outcome. Those comparisons are called unplanned (or a posteriori) comparisons.

Former example

We previously investigated whether the mean blood coagulation times for animals receiving different diets (A, B, C or D) were the same.

Imagine A is the standard diet, and we wish to compare each of diets B, C, D to diet A.

→→ planned comparisons!

After inspecting the treatment means, we find that A and D look similar, and B and C look similar, but A and D are quite different from B and C. We might want to formally test the hypothesis $\mu_A = \mu_D \neq \mu_B = \mu_C$.

→→ unplanned comparisons!
Another example

A plant physiologist recorded the length of pea sections grown in tissue culture with auxin present. The purpose of the experiment was to investigate the effects of various sugars on growth. Four different treatments were used, plus one control (no sugar):

- No sugar
- 2% glucose
- 2% fructose
- 1% glucose + 1% fructose
- 2% sucrose

Specific questions

The investigator wants to answer three specific questions:

- Does the addition of sugars have an effect on the lengths of the pea sections?
- Are there differences between the pure sugar treatments and the mixed sugar treatment?
- Are there differences among the pure sugar treatments?

Planned comparisons!
The plant physiologist’s data

ANOVA table

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between treatment</td>
<td>1077.3</td>
<td>4</td>
<td>269.3</td>
<td>49.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Within treatment</td>
<td>245.5</td>
<td>45</td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Unplanned comparisons

Suppose we are comparing $k$ treatment groups. Suppose ANOVA indicates that you reject $H_0 : \mu_1 = \cdots = \mu_k$

What next?
Which of the $\mu$’s are different from which others?

Consider testing $H_0 : \mu_i = \mu_j$ for all pairs $i,j$.

There are $\binom{k}{2} = \frac{k(k-1)}{2}$ such pairs.

- $k = 5 \quad \rightarrow \quad \binom{5}{2} = 10.$
- $k = 10 \quad \rightarrow \quad \binom{10}{2} = 45.$

Bonferroni correction

Suppose we have 10 treatment groups, and so 45 pairs.

If we perform 45 t-tests at the significance level $\alpha = 0.05$, we would expect to reject $5\% \times 45 \approx 2$ of them, even if all of the means were the same.

Let $\alpha = \Pr(\text{reject at least one pairwise test} \mid \text{all } \mu \text{'s the same}) \leq (\text{no. tests}) \times \Pr(\text{reject test #1} \mid \mu \text{'s the same})$

The Bonferroni correction:

Use $\alpha' = \alpha / (\text{no. tests})$ as the significance level for each test.

For example, with 10 groups and so 45 pairwise tests, we would use $\alpha' = 0.05 / 45 \approx 0.0011$ for each test.
Blood coagulation time

### Pairwise comparisons

<table>
<thead>
<tr>
<th>Comparison</th>
<th>p-value</th>
<th>( \alpha' = \frac{\alpha}{k} = \frac{0.05}{6} = 0.0083 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A vs B</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>A vs C</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>A vs D</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>B vs C</td>
<td>0.159</td>
<td></td>
</tr>
<tr>
<td>B vs D</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>C vs D</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>
The plant physiologist’s data

ANOVA table

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between treatment</td>
<td>1077.3</td>
<td>4</td>
<td>269.3</td>
<td>49.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Within treatment</td>
<td>245.5</td>
<td>45</td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( \binom{5}{2} = 10 \) pairwise comparisons \quad \rightarrow \quad \alpha’ = 0.05/10 = 0.005

For each pair, consider \( T_{i,j} = (\bar{Y}_i - \bar{Y}_j) / (\hat{\sigma} \sqrt{\frac{1}{n_i} + \frac{1}{n_j}}) \)

Use \( \hat{\sigma} = \sqrt{M_W} \quad (M_W = \text{within-group mean square}) \)
and refer to a t distribution with df = 45.
Results

\( \hat{\sigma} = 2.34 \)

\( n = 10 \) for each group

\[ SE = 2.34 \times \sqrt{2/10} = 1.05 \]

for each comparison.

\( df = 45, \ \alpha' = 0.005 \rightarrow t = 2.69 \)

Groups with

\[ |\bar{Y}_i - \bar{Y}_j| > 2.69 \times 1.05 = 2.81 \]

are deemed different.

Bonferroni-corrected CIs:

\[ (\bar{Y}_i - \bar{Y}_j) \pm 2.81 \]

Tukey’s HSD

HSD = “Honest significant difference”

Reject \( H_0 : \mu_i = \mu_j \) if

\[ |\bar{Y}_i - \bar{Y}_j| > Q_{\alpha}(k, df) \times \sqrt{M_W/n} \]

We’re assuming equal sample sizes for the treatment groups (n).

\( k = \) no. treatment groups; \( df = n \cdot k - k \)

\( Q_{\alpha}(k, df) = 1 - \alpha \) quantile of the “Studentized range distribution.”

We won’t go into where exactly \( Q_{\alpha}(k, df) \) comes from. Suffice it to say: it’s an adjustment not unlike the Bonferroni correction, and it can be calculated using \texttt{qtukey()} in R. Alternatively, the function \texttt{TukeyHSD()} will do the whole thing.
Results

Taking $\alpha = 0.05$, $k = 5$, $df = 45$,

$Q_\alpha(k, df) = 4.02.$

$\text{qtukey}(0.95, 5, 45)$

Groups with

$|\bar{Y}_i - \bar{Y}_j| > 4.02 \times \sqrt{5.46/10}$

$= 2.97$

are deemed different.

A comparison

Uncorrected:

Each interval, individually, had (in advance) a 95% chance of covering the true mean difference.

Corrected:

(In advance) there was a greater than 95% chance that all of the intervals would cover their respective parameters.
Newman-Keuls procedure

Goal: Identify sets of treatments whose mean responses are not significantly different.
(Assuming equal sample sizes for the treatment groups.)

Procedure:
1. Calculate the group sample means.
2. Order the sample means from smallest to largest.
3. Calculate a triangular table of all pairwise sample means.
4. Calculate $q_i = Q_{\alpha}(i, df)$ for $i = 2, 3, \ldots, k$. Use `qtukey()` in R!
5. Calculate $R_i = q_i \times \sqrt{M_W/n}$.

Newman-Keuls procedure (continued)

Procedure:
6. If the difference between the biggest and the smallest means is less than $R_k$, draw a line under all of the means and stop.
7. Compare the second biggest and the smallest (and the second-smallest and the biggest) to $R_{k-1}$. If observed difference is smaller than the critical value, draw a line between these means.
8. Continue to look at means for which a line connecting them has not yet been drawn, comparing the difference to $R_i$ with progressively smaller $i$'s.
Example

Sorted sample means:

<table>
<thead>
<tr>
<th></th>
<th>G+F</th>
<th>F</th>
<th>G</th>
<th>S</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>58.0</td>
<td>58.2</td>
<td>59.3</td>
<td>64.1</td>
<td>70.1</td>
</tr>
</tbody>
</table>

Table of differences:

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>G</th>
<th>S</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>G+F</td>
<td>0.2</td>
<td>1.3</td>
<td>6.1</td>
<td>12.1</td>
</tr>
<tr>
<td>F</td>
<td>1.1</td>
<td>5.9</td>
<td>11.9</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>4.8</td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td></td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Example (continued)

From the ANOVA table:

\[ M_W = 5.46 \quad n = 10 \text{ for each group} \quad \sqrt{M_W/10} = 0.739 \quad \text{df} = 45 \]

The \( q_i \) (using \( \text{df}=45 \) and \( \alpha = 0.05 \)):

\[
q_2 \quad q_3 \quad q_4 \quad q_5
\]
\[
2.85 \quad 3.43 \quad 3.77 \quad 4.02
\]

\[ R_i = q_i \times \sqrt{M_W/10}: \]

\[
R_2 \quad R_3 \quad R_4 \quad R_5
\]
\[
2.10 \quad 2.53 \quad 2.79 \quad 2.97
\]
Table of differences:

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>G</th>
<th>S</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>G+F</td>
<td>0.2</td>
<td>1.3</td>
<td>6.1</td>
<td>12.1</td>
</tr>
<tr>
<td>F</td>
<td>1.1</td>
<td>5.9</td>
<td>11.9</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>4.8</td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ R_i = q_i \times \sqrt{M_w/10} : \]

<table>
<thead>
<tr>
<th></th>
<th>R_2</th>
<th>R_3</th>
<th>R_4</th>
<th>R_5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.10</td>
<td>2.53</td>
<td>2.79</td>
<td>2.97</td>
</tr>
</tbody>
</table>

Results

Sorted sample means:

<table>
<thead>
<tr>
<th></th>
<th>G+F</th>
<th>F</th>
<th>G</th>
<th>S</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>58.0</td>
<td>58.2</td>
<td>59.3</td>
<td>64.1</td>
<td>70.1</td>
</tr>
</tbody>
</table>

Interpretation:

\[ G+F \approx F \approx G < S < C \]
Another example

Sorted sample means:

<table>
<thead>
<tr>
<th></th>
<th>D</th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.6</td>
<td>32.9</td>
<td>40.0</td>
<td>40.7</td>
<td>48.8</td>
<td></td>
</tr>
</tbody>
</table>

Table of differences:

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>3.3</td>
<td>10.4</td>
<td>11.1</td>
<td>19.2</td>
</tr>
<tr>
<td>C</td>
<td>7.1</td>
<td>7.8</td>
<td>15.9</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.7</td>
<td>8.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>8.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Example (continued)

From the ANOVA table:

\[ M_W = 21.29 \quad n = 4 \text{ for each group} \quad \sqrt{M_W/4} = 2.31 \quad df = 15 \]

The \( q_i \) (using df=15 and \( \alpha = 0.05 \)):

<table>
<thead>
<tr>
<th></th>
<th>( q_2 )</th>
<th>( q_3 )</th>
<th>( q_4 )</th>
<th>( q_5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.01</td>
<td>3.67</td>
<td>4.08</td>
<td>4.37</td>
</tr>
</tbody>
</table>

\[ R_i = q_i \times \sqrt{M_W/4} : \]

<table>
<thead>
<tr>
<th></th>
<th>( R_2 )</th>
<th>( R_3 )</th>
<th>( R_4 )</th>
<th>( R_5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.95</td>
<td>8.47</td>
<td>9.40</td>
<td>10.07</td>
</tr>
</tbody>
</table>
Example (continued)

Table of differences:

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>3.3</td>
<td>10.4</td>
<td>11.1</td>
<td>19.2</td>
</tr>
<tr>
<td>C</td>
<td>7.1</td>
<td>7.8</td>
<td>15.9</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.7</td>
<td>8.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>8.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ R_i = q_i \times \sqrt{\frac{M_w}{4}}: \]

\[
\begin{array}{cccc}
R_2 & R_3 & R_4 & R_5 \\
6.95 & 8.47 & 9.40 & 10.07 \\
\end{array}
\]

Results

Sorted sample means:

<table>
<thead>
<tr>
<th></th>
<th>D</th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>29.6</td>
<td>32.9</td>
<td>40.0</td>
<td>40.7</td>
<td>48.8</td>
</tr>
</tbody>
</table>

Interpretation:

\{D, C, A, B\} < E \quad \text{and} \quad D < \{A, B\}
For the Tukey and Newman-Keuls methods, we assumed that the numbers of responses in each treatment group were the same.

What to do if they vary?

- If they don’t vary too much, use $1/n_i + 1/n_j$ in place of $2/n$.

- If they are quite different, it’s probably best to just stick with the Bonferroni correction.