One treatment factor

- fixed effects, global test, contrasts, ...
- random effects, variance components, ...
- factorial treatment structure (fixed effects), two-way ANOVA (or more factors), concept of interaction, $2^k$-designs, ...

Multiple treatment factors

- random effects, mixed effects models, nested factor structure, ...
- split-plot, split-split plot designs, different models on whole- and subplots, ...
- RCB with factorial treatment structure, ...

Experimental units

- homogeneous
- inhomogeneous

CRD

Block Designs

- one block f. two (more)

RCB

- (B)IBD
- Latin Squares
- Youden Squares

Block Designs

- block size
  - large
  - small

Experimental units

- Block Designs
  - one block f. two (more)
    - block size
      - large
      - small
Factorial Treatment Structure

- So far (in CRDs), the treatments had no “structure”.

- So called factorial treatment structure exists if the $g$ treatments are the combination of the levels of two or more factors.

- In the case that we see all the possible combinations of the levels of the two factors, we call the factors crossed.

- Examples
  - Biomass of crop: different fertilizers and different crop varieties.
  - Battery life: Different temperature levels and different plate material (Montgomery, 1991, Example 7-3.1).
  - ...
Example (Linder, A. und W. Berchtold, 1982)

- Response: Needleweight of 20 three-week old pine seedlings [in 1/100 g].

- Two factors:
  - \( A \): “origin” with levels \{Taglieda, Pfyn, Rheinau\}
  - \( B \): “exposure to light” with levels \{short, long, permanent\}

- We denote by \( y_{ijk} \) the \( k \)th response of the treatment formed by the \( i \)th level of factor \( A \) and the \( j \)th level of factor \( B \).
Two Factor Design: Generic Data Table

<table>
<thead>
<tr>
<th></th>
<th>$B_1$</th>
<th>$B_2$</th>
<th>$B_3$</th>
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<tr>
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<td>...</td>
</tr>
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<td>$y_{123}$</td>
<td>$y_{133}$</td>
<td>...</td>
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<tr>
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<td>$y_{124}$</td>
<td>$y_{134}$</td>
<td>...</td>
</tr>
<tr>
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<td>$y_{221}$</td>
<td>$y_{231}$</td>
<td>...</td>
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<td>...</td>
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</table>
## Data Table of Our Example

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<tr>
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<tr>
<td></td>
<td>50</td>
<td>62</td>
<td>95</td>
</tr>
</tbody>
</table>
Visualization

- As for one-way ANOVA situation
  - for all treatment combinations
  - factor-wise summaries (“marginal summaries”)

- More useful: Interaction plot (see R-code)
Factorial Treatment Structure

- The **structure of the treatment** influences the **analysis** of the data.

- Setup:
  - Factor $A$ with $a$ levels
  - Factor $B$ with $b$ levels
  - $n$ replicates for **every** combination → a so called **balanced design**
  - Total of $N = a \cdot b \cdot n$ observations

- We could analyze this with the usual **cell means model** (ignoring the special treatment structure).

- Typically, we have research questions about **both** factors and their possible interaction (interplay).
Factorial Treatment Structure

- **Examples:**
  - “Is effect of light exposure location specific?”
    - (→ **interaction** between light exposure and location)
  - “What is the effect of light exposure averaged over all locations?”
    - (→ **main effect** of light exposure)
  - “What is the effect of location averaged over all exposure levels?”
    - (→ **main effect** of location)

- We could use the cell means (one-way ANOVA) model and try to answer these questions with **appropriate contrasts** (→ complicated).

- Easier: Use a model that incorporates the **factorial structure** of the treatments.
Factorial Model

The two-way ANOVA model with interaction is

\[ Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk} \]

where

- \( \alpha_i \) is the main effect of factor A at level \( i \).
- \( \beta_j \) is the main effect of factor B at level \( j \).
- \( (\alpha\beta)_{ij} \) is the interaction effect between A and B for level combination \( i, j \) (not the product \( \alpha_i\beta_j \)!)  
- \( \epsilon_{ijk} \) are i.i.d. \( N(0, \sigma^2) \) errors.
- Typically, sum-to-zero constraints are being used, i.e.
  - \( \sum_{i=1}^{a} \alpha_i = 0, \sum_{j=1}^{b} \beta_j = 0 \) \( \rightarrow \) \( a - 1 \) and \( b - 1 \) degrees of freedom
  - \( \sum_{i=1}^{a}(\alpha\beta)_{ij} = 0, \sum_{j=1}^{b}(\alpha\beta)_{ij} = 0 \) \( \rightarrow \) \((a - 1) \cdot (b - 1)\) degrees of freedom

because two factors involved
Interpretation of Main Effects

- **Main effects** are nothing else than the **average effect** when moving from row to row (column to column).

- **Interaction effect** is the difference to the main effects model, i.e. it measures how far the treatment means differ from the main effects model.

- If there is **no interaction**, the effects are **additive**.

- In our example it would mean: “No matter what location we are considering, the effect of light exposure is always the same.”
Visualization of Model

Factor $A$, Factor $B$ with two levels each

$$E[Y_{ijk}] = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij}$$

- Effect of $(B_1 \rightarrow B_2)$ at $A_1$
  - $E[Y] = \mu + \alpha_1 + \beta_2 + (\alpha\beta)_{12}$

- Effect of $(A_1 \rightarrow A_2)$ at $B_1$
  - $\mu + \alpha_2 + \beta_1 + (\alpha\beta)_{21}$

- Effect of $(A_1 \rightarrow A_2)$ at $B_2$
  - $\mu + \alpha_2 + \beta_2 + (\alpha\beta)_{22}$
Visualization of Model

If no interaction is present, lines will be parallel.

Interaction plot is nothing else than empirical version of this plot.

\[
E[Y_{ijk}] = \mu + \alpha_i + \beta_j
\]
Two-Way ANOVA Step By Step

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<td>ε₃₃₁</td>
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**Parameter Estimates**

- Estimates for the balanced case (and the sum-to-zero constraints) are

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimator</th>
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<tr>
<td>$\mu$</td>
<td>$\hat{\mu} = \bar{y}_{..}$</td>
</tr>
<tr>
<td>$\alpha_i$</td>
<td>$\hat{\alpha}<em>i = \bar{y}</em>{i..} - \bar{y}_{..}$</td>
</tr>
<tr>
<td>$\beta_j$</td>
<td>$\hat{\beta}<em>j = \bar{y}</em>{.j} - \bar{y}_{..}$</td>
</tr>
<tr>
<td>$(\alpha\beta)_{ij}$</td>
<td>$(\hat{\alpha}\beta)<em>{ij} = \bar{y}</em>{ij} - \hat{\mu} - \hat{\alpha}_i - \hat{\beta}_j$</td>
</tr>
</tbody>
</table>

- This means: we estimate the main effects as if the other factors wouldn’t be there (see next slides).
Main Effect of Location

- Original data-set

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<td>95</td>
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</table>

- Ignore factor “exposure”, estimate effect as in one-way ANOVA model

$\rightarrow \hat{\alpha}_i$
Main Effect of Light Exposure

- Original data-set

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<td>95</td>
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</tr>
</tbody>
</table>

- Ignore factor “location”, estimate effect as in one-way ANOVA model

\[ \hat{\beta}_j \]
Sum of Squares

- Again, total sum of squares can be **partitioned** into different sources, i.e.
  \[ SS_T = SS_A + SS_B + SS_{AB} + SS_E. \]

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of squares (SS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>(a - 1)</td>
<td>[\sum_{i=1}^{a} b \cdot n \cdot \hat{\alpha}_i^2]</td>
</tr>
<tr>
<td>B</td>
<td>(b - 1)</td>
<td>[\sum_{j=1}^{b} a \cdot n \cdot \hat{\beta}_j^2]</td>
</tr>
<tr>
<td>AB</td>
<td>((a - 1) \cdot (b - 1))</td>
<td>[\sum_{i=1}^{a} \sum_{j=1}^{b} n \cdot (\hat{\alpha} \hat{\beta})_{ij}^2]</td>
</tr>
<tr>
<td>Error</td>
<td>((n - 1) \cdot ab)</td>
<td>[\sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{k=1}^{n} (y_{ijk} - \bar{y}_{ij})^2]</td>
</tr>
<tr>
<td>Total</td>
<td>(abn - 1)</td>
<td>[\sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{k=1}^{n} (y_{ijk} - \bar{y}_{..})^2]</td>
</tr>
</tbody>
</table>

- **Squared effect**: 
  \[\sum_{i=1}^{a} b \cdot n \cdot \hat{\alpha}_i^2\]

- **# observations with that effect**

- **Fitted value**: 
  \[\sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{k=1}^{n} (y_{ijk} - \bar{y}_{ij})^2\]

- **Product of df's of involved factors**

- **#observations – 1 – sum(df above)**

- **Degrees of freedom of total**
As before, we can construct an **ANOVA table**

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$</td>
<td>$a - 1$</td>
<td>$SS_A$</td>
<td>$\frac{SS_A}{a - 1}$</td>
<td>$\frac{MS_A}{MS_E}$</td>
</tr>
<tr>
<td>$B$</td>
<td>$b - 1$</td>
<td>$SS_B$</td>
<td>$\frac{SS_B}{b - 1}$</td>
<td>$\frac{MS_B}{MS_E}$</td>
</tr>
<tr>
<td>$AB$</td>
<td>$(a - 1) \cdot (b - 1)$</td>
<td>$SS_{AB}$</td>
<td>$\frac{SS_{AB}}{(a - 1)(b - 1)}$</td>
<td>$\frac{MS_{AB}}{MS_E}$</td>
</tr>
<tr>
<td>Error</td>
<td>$ab \cdot (n - 1)$</td>
<td>$SS_E$</td>
<td>$\frac{SS_E}{(n - 1)ab}$</td>
<td>$\frac{MS_E}{MS_E}$</td>
</tr>
</tbody>
</table>

Under the corresponding (global) null-hypothesis, the $F$-ratio is again $F$-distributed with degrees of freedom defined through the numerator and the denominator, respectively.
**F-Tests: Overview**

- **Interaction $AB$**
  - $H_0: (\alpha\beta)_{ij} = 0$ for all $i, j$
  - $H_A$: At least one $(\alpha\beta)_{ij} \neq 0$
  - Under $H_0$: \( \frac{MS_{AB}}{MS_E} \sim F_{(a-1)(b-1), ab(n-1)} \)

- **Main effect $A$**
  - $H_0: \alpha_i = 0$ for all $i$
  - $H_A$: At least one $\alpha_i \neq 0$
  - Under $H_0$: \( \frac{MS_A}{MS_E} \sim F_{(a-1), ab(n-1)} \)

- **Main effect $B$**
  - $H_0: \beta_j = 0$ for all $j$
  - $H_A$: At least one $\beta_j \neq 0$
  - Under $H_0$: \( \frac{MS_B}{MS_E} \sim F_{(b-1), ab(n-1)} \)
Analyzing the ANOVA Table

- Typically, the $F$-tests are analyzed from **bottom to top** (in the ANOVA table).

- Here, this means we start with the $F$-test of the interaction.

- If we reject:
  - Conclude that we need an interaction in our model, i.e. the effect of $A$ depends on the level of $B$.
  - **Don’t continue** testing the main-effects (**principle of hierarchy**)
  - Perform individual analysis for every level of factor $A$ (or $B$), error of **full model** can be re-used (see later).
  - Have a look at **interaction plot** to get a better understanding of what is going on.
  - Interaction might be based on a **single** cell.
  - Transformation might help in getting rid of the interaction.

- If we can’t reject, continue testing the main effects.
Two-Way ANOVA model in R

- Define model with interaction in formula interface
  
  ```r
  > fit <- aov(y ~ location * exposure, data = data)
  > summary(fit)
  
  Df  Sum Sq  Mean Sq   F value  Pr(>F)
  location      2  2053.1  1026.4  69.981   3.28e-06  ***
  exposure      2  4074.1  2037.1 138.890  1.72e-07  ***
  location:exposure  4   183.7   45.7   3.117   0.0722 .
  Residuals     9    132.0   14.7
  ---
  Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
  
  ```

- We can also use the following equivalent notation
  
  ```r
  > fit <- aov(y ~ location + exposure + location:exposure, data = data) ## equivalent version
  ```
More than Two Factors

- Model can be easily extended to **more than two** factors, e.g. $A, B, C$.

- Model then includes higher-order interactions:
  \[
  Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \\
  (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + \\
  (\alpha\beta\gamma)_{ijk} + \\
  \epsilon_{ijkl}
  \]

  with $\epsilon_{ijkl}$ i.i.d. $N(0, \sigma^2)$ and the usual sum-to-zero constraints.

- Two-way interaction describes how a main-effect depends on the level of the other factor.

- Three-way interaction describes how a two-way interaction depends on level of third factor …
Interpretation

- The higher-order interactions are quite difficult to interpret.
- Parameter estimation as before.
- Degrees of freedom of an interaction is the product of the df’s of the involved factors (as usual).
- E.g., for the three-way interaction:
  \[(a - 1) \cdot (b - 1) \cdot (c - 1)\]