2 Two-way ANOVA

In the one-way design there is only one factor. What if there are several factors? Often, we are interested to know the simultaneous effects of multiple factors, e.g., gender and smoking on hypertension. The statistical approach to analyze data from a two-way design is two-way ANOVA.

Iron retention. An experiment was performed to determine the factors that affect iron retention. The experiment was done on 108 mice, which were randomly divided into 6 groups of 18 each. These groups consist of the six combinations of two forms of iron and three concentrations.

The table below shows the mean logged retention percentage.

<table>
<thead>
<tr>
<th>Form</th>
<th>low</th>
<th>medium</th>
<th>high</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe3+</td>
<td>2.28 (Y_{11.})</td>
<td>1.90 (Y_{12.})</td>
<td>1.16 (Y_{13.})</td>
</tr>
<tr>
<td>Fe2+</td>
<td>2.40 (Y_{21.})</td>
<td>2.09 (Y_{22.})</td>
<td>1.68 (Y_{23.})</td>
</tr>
</tbody>
</table>

The figure below shows the mean retention levels (log).

Several scientific questions:
(1) Are the means different by iron form?
(2) Are the means different by dosage?
(3) Does difference in the means between the two iron forms change as a function of dosage?

The first two questions can be answered by one-way ANOVA. Here we use the two-way anova to answer all the questions simultaneously.

2.1 The two-way layout

A two-way layout is an experiment design involving two factors, each at two or more levels. If there are I levels of factor A and J of factor B, there are \( I \times J \) combinations. We assume that \( K \) independent observations are taken for each of the \( IJ \) combinations.

Let \( Y_{ijk} \) denote the \( k \)th observation in cell \( ij \). The statistical model is a natural extension of the one-way anova:

\[
Y_{ijk} = \mu + \alpha_i + \beta_j + \delta_{ij} + \epsilon_{ijk}, \text{ with } \epsilon_{ijk} \sim N(0, \sigma^2)
\]

with the constraints

\[
\sum_{i=1}^{I} \alpha_i = 0 \quad \sum_{j=1}^{J} \beta_j = 0 \quad \sum_{i=1}^{I} \delta_{ij} = \sum_{j=1}^{J} \delta_{ij} = 0
\]

Interpretations of the parameters:

\( \mu \) is the grand mean over all cells
\( \alpha_i \) is the main effect of the \( i \)th level of factor A
\( \beta_j \) is the main effect of the \( j \)th level of factor B
\( \delta_{ij} \) is the interaction effect between the \( i \)th level of factor A and the \( j \)th level of factor B, i.e., it denotes the effect of combining the \( i \)th level of factor A and the \( j \)th level of factor B.

under the two-way layout,
the mean response in the $i$th level of factor A is $\mu + \alpha_i$
the mean response in the $j$th level of factor B is $\mu + \beta_j$
the mean response in the cell $ij$ is $\mu + \alpha_i + \beta_j + \delta_{ij}$
We say the two factors have an interaction effect on response if any of $\delta_{ij}$ is not zero.
When there is no interaction, the means are

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<tbody>
<tr>
<td>Fe2+</td>
<td>$\mu + \alpha_1 + \beta_1$</td>
<td>$\mu + \alpha_1 + \beta_2$</td>
<td>$\mu + \alpha_1 + \beta_3$</td>
</tr>
<tr>
<td>Fe3+</td>
<td>$\mu + \alpha_2 + \beta_1$</td>
<td>$\mu + \alpha_2 + \beta_2$</td>
<td>$\mu + \alpha_2 + \beta_3$</td>
</tr>
<tr>
<td>Difference</td>
<td>$\alpha_1 - \alpha_2$</td>
<td>$\alpha_1 - \alpha_2$</td>
<td>$\alpha_1 - \alpha_2$</td>
</tr>
</tbody>
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The graph of the means would look like

2.2 Two-way ANOVA

When there is an interaction between iron form and dosage, the means are

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</thead>
<tbody>
<tr>
<td>Fe3+</td>
<td>$\mu + \alpha_1 + \beta_1 + \delta_{11}$</td>
<td>$\mu + \alpha_1 + \beta_2 + \delta_{12}$</td>
<td>$\mu + \alpha_1 + \beta_3 + \delta_{13}$</td>
</tr>
<tr>
<td>Fe2+</td>
<td>$\mu + \alpha_2 + \beta_1 + \delta_{12}$</td>
<td>$\mu + \alpha_2 + \beta_2 + \delta_{22}$</td>
<td>$\mu + \alpha_2 + \beta_3 + \delta_{23}$</td>
</tr>
<tr>
<td>Difference</td>
<td>$\alpha_1 - \alpha_2 + (\delta_{11} - \delta_{21})$</td>
<td>$\alpha_1 - \alpha_2 + (\delta_{12} - \delta_{22})$</td>
<td>$\alpha_1 - \alpha_2 + (\delta_{13} - \delta_{23})$</td>
</tr>
</tbody>
</table>

The graph of the means would look like
2.2.1 Notations

\[
\begin{align*}
\bar{Y}_{\text{tot}} &= \sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} \frac{Y_{ijk}}{(IJK)} \\
\bar{Y}_{i\cdot} &= \sum_{j=1}^{J} \sum_{k=1}^{K} \frac{Y_{ijk}}{(JK)} \\
\bar{Y}_{\cdot j} &= \sum_{i=1}^{I} \sum_{k=1}^{K} \frac{Y_{ijk}}{(IK)} \\
\bar{Y}_{ij} &= \sum_{k=1}^{K} \frac{Y_{ijk}}{K}
\end{align*}
\]

\[
Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \epsilon_{ijk}, \quad \epsilon_{ijk} \overset{iid}{\sim} N(0, \sigma^2), \quad \sum_i \alpha_i = \sum_j \beta_j = \sum_{i,j} \gamma_{ij} = \sum_{i,j} \gamma_{ij} = 0
\]

Assume that the observations are independent and normally distributed with equal variance, the log likelihood is

\[
l = -\frac{IJK}{2} \log(2\pi\sigma^2) - \frac{1}{2\sigma^2} \sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} (Y_{ijk} - \mu - \alpha_i - \beta_j - \delta_{ij})^2
\]

The mles are

\[
\begin{align*}
\hat{\mu} &= \bar{Y}_{\text{tot}} \\
\hat{\alpha}_i &= \bar{Y}_{i\cdot} - \bar{Y}_{\cdot\cdot}, \quad i = 1, \ldots, I \\
\hat{\beta}_j &= \bar{Y}_{\cdot j} - \bar{Y}_{\cdot\cdot}, \quad j = 1, \ldots, J \\
\hat{\delta}_{ij} &= \bar{Y}_{ij} - \bar{Y}_{i\cdot} - \bar{Y}_{\cdot j} + \bar{Y}_{\cdot\cdot}
\end{align*}
\]

Similar to the definition used in the one-way ANOVA, the sum of squares of total is
defined as

\[
SSTO = \sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} (Y_{ijk} - \bar{Y}_{ijk})^2
= \sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} (Y_{ijk} - \bar{Y})^2
\]

\(SSTO\) can be decomposed to

\[SSTO = SSA + SSB + SSAB + SSE\]

where

\[
SSA = JK \sum_{i=1}^{I} (\bar{Y}_{i.} - \bar{Y}_{..})^2
= JK \sum_{i=1}^{I} \hat{\alpha}_i^2
\]

\[
SSB = IK \sum_{j=1}^{J} (\bar{Y}_{.j} - \bar{Y}_{..})^2
= IK \sum_{j=1}^{J} \hat{\beta}_j^2
\]

\[
SSAB = K \sum_{i=1}^{I} \sum_{j=1}^{J} (\bar{Y}_{ij} - \bar{Y}_{i..} - \bar{Y}_{..j} + \bar{Y})^2
= K \sum_{i=1}^{I} \sum_{j=1}^{J} \hat{\delta}_{ij}^2
\]

\[
SSE = \sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} (Y_{ijk} - \bar{Y}_{ij})^2
= (K - 1) \sum_{i} \sum_{j} S^2_{ij}
= \sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} (Y_{ijk} - (\bar{\mu} + \hat{\alpha}_i + \hat{\beta}_j + \hat{\delta}_{ij}))^2
\]

The proof is also similar to that of one-way ANOVA. Essentially, we need to use the
\begin{align*}
Y_{ijk} - \bar{Y}_- = (Y_{ijk} - \bar{Y}_{ij}) + (Y_{ij} - \bar{Y}_j) + (Y_{ij} - \bar{Y}_j) + (Y_{ij} - \bar{Y}_j) + (Y_{ij} - \bar{Y}_j) + (Y_{ij} - \bar{Y}_j) + \bar{Y}_j + \bar{Y}_j
\end{align*}

**Theorem A: Expectations of Sums of Squares** Under the two-way ANOVA model,

1. \( E(MSE) = E(SSE/[IJ(K-1)]) - \sigma^2 \)
2. \( E(MSA) = E(SSA/(I-1)) - \sigma^2 + \frac{JK}{I-1} \sum_{i=1}^{I} \alpha_i^2 \)
3. \( E(MSR) = E(SSR/(J-1)) - \sigma^2 + \frac{IK}{J-1} \sum_{j=1}^{J} \beta_j^2 \)
4. \( E(MSAB) = E(SSAB/[(I-1)(J-1)]) - \sigma^2 + \frac{K}{(I-1)(J-1)} \sum_{i=1}^{I} \sum_{j=1}^{J} \delta_{ij}^2 \)

Proof: They can be proved by using Lemma A:

Let \( X_i \), where \( i = 1, \ldots, n \) be independent random variables with \( E(X_i) = \mu_i \) and \( \text{Var}(X_i) = \sigma^2 \). Then

\[ E(X_i - \bar{X})^2 = (\mu_i - \bar{\mu})^2 + \frac{n-1}{n} \sigma^2 \]

Apply it to (1): \( E(Y_{ijk} - \bar{Y}_{ij})^2 = (0 - 0)^2 + (K-1)/K \sigma^2 \) thus

\[ E(SSE) = E[\sum_{ij} (K-1)[S_{ij}^2] = IJK[E(S_{ij}^2)] = IJK(K-1) \sigma^2 \]

Apply it (2):

Apply it to (3):

Notice that \( Y_{ijk} \sim N(\mu + \alpha_i + \beta_j + \delta_{ij}, \sigma^2) \) and apply the lemma to SSTO.