Correspondence between Experimental Designs and Mixed-Effect Models
Experimental Design Terminology

Experiment – An investigation in which the investigator applies some treatments to experimental units and then observes the effect of the treatments on the experimental units by measuring one or more response variables.
Treatment – a condition or set of conditions applied to experimental units in an experiment.

Experimental Unit - the physical entity to which a treatment is randomly assigned and independently applied.
Response Variable – a characteristic of an experimental unit that is measured after treatment and analyzed to assess the effects of treatments on experimental units.

Observational Unit - the unit on which a response variable is measured. There is often a one-to-one correspondence between experimental units and observational units, but that is not always true.
• In our example with genotypes, trays, and seedlings, trays were the experimental units because genotypes were randomly assigned to trays.

• Seedlings were the observational units because the response was measured separately for each seedling.

• Whenever there is more than one observational unit for an experimental unit or whenever the response is measured multiple times for an experimental unit, we say we have "multiple observations per experiment unit." This scenario is also referred to as "subsampling" or "pseudo-replication."
• Whenever an experiment involves multiple observations per experimental unit, it is important to include a random effect for each experimental unit.

• Without a random effect for each experimental unit, a one-to-one correspondence between observations and experimental units is assumed.

• Including random effects in a model is one way to account for a lack of independence among observations that might be expected based on the design of experiment.
Suppose we have four litters of four mice each.
Suppose we randomly assign four treatments to the mice in each litter.
Suppose we obtain two replicate muscle samples from each mouse and measure the response for each sample.
Let $Y_{ijk} = \mu + \tau_i + \epsilon_{ij} + M_{ij} + \varepsilon_{ijk}$, where $Y_{ijk}$ is the $k$th measurement of the response for the mouse from litter $j$ that received treatment $i$, $(i=1,2,3,4; j=1,2,3,4; k=1,2)$. 

$$\beta = \begin{bmatrix} \mu \\ \tau_1 \\ \tau_2 \\ \tau_3 \\ \tau_4 \end{bmatrix} \in \mathbb{R}^5$$ is an unknown vector of fixed parameters.

$$\varepsilon = \begin{bmatrix} \varepsilon_{11} \\ \varepsilon_{12} \\ \varepsilon_{13} \\ \varepsilon_{14} \\ \varepsilon_{21} \\ \varepsilon_{22} \\ \varepsilon_{23} \\ \varepsilon_{24} \\ \varepsilon_{31} \\ \varepsilon_{32} \\ \varepsilon_{33} \\ \varepsilon_{34} \\ \varepsilon_{41} \\ \varepsilon_{42} \\ \varepsilon_{43} \\ \varepsilon_{44} \\ \varepsilon_{51} \\ \varepsilon_{52} \\ \varepsilon_{53} \\ \varepsilon_{54} \\ \varepsilon_{61} \\ \varepsilon_{62} \\ \varepsilon_{63} \\ \varepsilon_{64} \\ \varepsilon_{71} \\ \varepsilon_{72} \\ \varepsilon_{73} \\ \varepsilon_{74} \\ \varepsilon_{81} \\ \varepsilon_{82} \\ \varepsilon_{83} \\ \varepsilon_{84} \\ \varepsilon_{91} \\ \varepsilon_{92} \\ \varepsilon_{93} \\ \varepsilon_{94} \\ \varepsilon_{101} \\ \varepsilon_{102} \\ \varepsilon_{103} \\ \varepsilon_{104} \end{bmatrix}'$$ is a vector of random effects.
\[
\mathbf{e} = [e_{111}, e_{112}, e_{211}, e_{212}, \ldots, e_{411}, e_{412}, \ldots, e_{441}, e_{442}]
\]

is a vector of random errors.

With \[
\mathbf{y} = [y_{111}, y_{112}, y_{211}, y_{212}, \ldots, y_{411}, y_{412}, \ldots, y_{441}, y_{442}]
\]
we can write the model as a linear mixed effects model

\[
\mathbf{y} = X \mathbf{\beta} + Z \mathbf{\alpha} + \mathbf{e}, \quad \text{where}
\]
The matrix above repeated 3 more times.
We can write less and be more precise using Kronecker product notation.

\[
X = \frac{1}{\sim} \otimes \left[ \frac{1}{\sim} \otimes \begin{array}{c} 1 \otimes 1 \\ \frac{1}{8 \times 1} \otimes 1 \otimes 1 \\ \frac{1}{4 \times 4} \otimes 1 \otimes 1 \\ \frac{1}{2 \times 1} \otimes 1 \otimes 1 \end{array} \right] \quad Z = \left[ \begin{array}{c} \frac{1}{4 \times 4} \otimes 1 \otimes 1 \\ \frac{1}{8 \times 1} \otimes 1 \otimes 1 \\ \frac{1}{16 \times 16} \otimes 1 \otimes 1 \end{array} \right]
\]

In this experiment, we have two random factors: Litter and Mouse.

We can partition our random effects vector \( \sim \) into a vector of litter effects and
a vector of mouse effects:

\[ u \sim \left[ \begin{array}{c} l \\ m \end{array} \right] \sim \left[ \begin{array}{c} l_1 \\ l_2 \\ l_3 \\ l_4 \end{array} \right] \sim \left[ \begin{array}{c} m_{11} \\ m_{21} \\ m_{31} \\ m_{41} \\ m_{12} \\ \vdots \\ m_{44} \end{array} \right] \]

We make the usual assumption that

\[ u \sim \left[ \begin{array}{c} l \\ m \end{array} \right] \sim N\left( \left[ \begin{array}{c} 0 \\ 0 \end{array} \right], \left[ \begin{array}{cc} \sigma_l^2 I & 0 \\ 0 & \sigma_m^2 I \end{array} \right] \right) \]

where \( \sigma_l^2, \sigma_m^2 \in \mathbb{R}^+ \) are unknown parameters.
We can partition

\[
Z = \begin{bmatrix}
\frac{I \otimes 1}{4 \times 4} & \frac{I \otimes 1}{8 \times 1} \\
\frac{I \otimes 1}{16 \times 16} & \frac{1}{2 \times 1}
\end{bmatrix}
\]

\[
= \begin{bmatrix}
Z_L & Z_M
\end{bmatrix}
\]

We have \( Z U = \begin{bmatrix} Z_L, Z_M \end{bmatrix} \begin{bmatrix} \ell \\ m \end{bmatrix} \)

\[
= Z_L \ell + Z_M m \quad \text{and}
\]
\[ \text{Var}(z_y) = z G z' \]

\[ = \begin{bmatrix} z_e & z_m \end{bmatrix} \begin{bmatrix} \sigma_e^2 I & 0 \\ 0 & \sigma_m^2 I \end{bmatrix} \begin{bmatrix} z'_e \\ z'_m \end{bmatrix} \]

\[ = z_e (\sigma_e^2 I) z'_e + z_m (\sigma_m^2 I) z'_m \]

\[ = \sigma_e^2 z_e z'_e + \sigma_m^2 z_m z'_m \]

\[ = \sigma_e^2 \begin{bmatrix} 4 \times 4 \end{bmatrix} \otimes \begin{bmatrix} 11' \\ 8 \times 8 \end{bmatrix} + \sigma_m^2 \begin{bmatrix} 16 \times 16 \end{bmatrix} \otimes \begin{bmatrix} 11' \\ 2 \times 2 \end{bmatrix} \]
We usually assume that all random effects and random errors are mutually independent and that the errors (like the effects within each factor) are identically distributed:

\[
\begin{bmatrix}
  \epsilon \\
  m \\
  e
\end{bmatrix} \sim N \left( \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix}
  \sigma^2_e I & 0 & 0 \\
  0 & \sigma^2_m I & 0 \\
  0 & 0 & \sigma^2_e I
\end{bmatrix} \right)
\]

The unknown variance parameters \( \sigma^2_e, \sigma^2_m, \sigma^2_e \in \mathbb{R}^+ \) are called variance components.
In this case, we have $R = \text{Var}(\varepsilon) = \sigma^2_e \mathbf{I}$.

Thus, $\text{Var}(y) = Z G Z' + R$

$$= \sigma^2_x Z_x Z_x' + \sigma^2_m Z_m Z_m' + \sigma^2_e \mathbf{I}.$$ 

This is a block diagonal matrix with blocks as follows.

\[
\begin{pmatrix}
\text{To get it to fit on one slide} \\
\text{let } a = \sigma^2_x, b = \sigma^2_m, c = \sigma^2_e
\end{pmatrix}
\]
\[
\begin{array}{cccccccc}
  a + b + c & a + b & a & a & a & a & a & a \\
  a + b & a + b + c & a & a & a & a & a & a \\
  a & a & a + b + c & a + b & a & a & a & a \\
  a & a & a + b & a + b + c & a & a & a & a \\
  a & a & a & a + b + c & a + b & a & a & a \\
  a & a & a & a + b & a + b + c & a & a & a \\
  a & a & a & a & a + b + c & a + b & a & a \\
  a & a & a & a & a & a + b & a + b + c & a \\
\end{array}
\]
The random effects specify the correlation structure in the data
Without the mouse random effects, our model would correspond to an RCBD with 2 mice per treatment per litter.
With no random effects, our model would correspond to a CRD with 8 mice per treatment.
## Split-Plot Experimental Designs

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Note: Split Plot or Sub Plot

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Split-Plot Experimental Designs

• This experiment has two factors: genotype and fertilizer amount.

• Genotype has levels A, B, and C.

• Fertilizer has levels 0, 50, 100, 150 lbs. N / acre.

• Genotype is called the *whole-plot factor* because its levels are randomly assigned to whole plots.

• Fertilizer is called the *split-plot factor* because its levels are randomly assigned to split plots within each whole plot.
Definition of Experimental Units in Split-Plot Designs

- Plots are the *whole-plot experimental units* because the levels of the whole-plot factor (genotype) are randomly assigned to plots.

- The split-plots are the *split-plot experimental units* because the levels of the split-plot factor (amount of fertilizer) are randomly assigned to split plots within each whole plot.

- Thus we have two different sizes of experimental units in split-plot experimental designs.
**Same Treatment Structure in a RCBD**

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**Same Treatment Structure in a CRD**

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Why use a split-plot design?

Consider a split-plot design if

1. Comparisons among the levels of the split-plot factor are of greater interest than comparisons among the levels of the whole-plot factor.

2. Logistical constraints make a CRD or RCBD impractical.
## Split-Plot Experimental Design

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Split-plot design structures don’t necessarily involve plots in the usual sense.

• Suppose eight pairs of mice from eight litters are housed in eight cages so that each cage holds two mice from the same litter.

• Suppose diets 1 and 2 are randomly assigned to the litters with four litters per diet.

• Within each cage, suppose drugs 1 and 2 are randomly assigned to the mice with one mouse per drug.
Conceptual Picture of the Experiment
Split-plot design structures don’t necessarily involve plots in the usual sense.

- Diet is the whole-plot treatment factor.
- Litters are the whole-plot experiment units.
- Drug is the split-plot treatment factor.
- Mice are the split-plot experiment units.
\[ Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \epsilon_{ijk} \quad (i=1,2; \ j=1,2; \ k=1,...,4) \]

\[ \mathbf{y} = \begin{bmatrix} y_{111} \\ y_{121} \\ y_{112} \\ y_{122} \\ y_{113} \\ y_{123} \\ y_{114} \\ y_{124} \\ y_{211} \\ y_{221} \\ y_{212} \\ y_{222} \\ y_{213} \\ y_{223} \\ y_{214} \\ y_{224} \end{bmatrix} \]

\[ \mathbf{\alpha} = \begin{bmatrix} \alpha_1 \\ \alpha_2 \\ \beta_1 \\ \beta_2 \end{bmatrix} \]

\[ \mathbf{u} = \begin{bmatrix} l_{11} \\ l_{12} \\ l_{13} \\ l_{14} \\ l_{21} \\ l_{22} \\ l_{23} \\ l_{24} \end{bmatrix} \]

\[ \mathbf{e} = \begin{bmatrix} e_{111} \\ e_{121} \\ \vdots \\ \vdots \\ \vdots \\ e_{224} \end{bmatrix} \]
\[ X = \begin{bmatrix} \frac{1}{16} \mathbb{1} & \frac{1}{2} \mathbb{1} & \frac{1}{8} \mathbb{1} & \frac{1}{2} \mathbb{1} & \frac{1}{4} \mathbb{1} \end{bmatrix} \]

\[ Z = \frac{1}{8 \times 8} \mathbb{1} \mathbb{1} \]

\[ Y = X \beta + Z \gamma + \epsilon \]
\[
\begin{bmatrix}
u \\
\epsilon
\end{bmatrix} \sim N\left(\begin{bmatrix}0 \\
0
\end{bmatrix}, \begin{bmatrix}
\sigma_e^2 I & 0 \\
0 & \sigma_e^2 I
\end{bmatrix}\right)
\]

\[
\text{Var}(Zu) = ZGZ' = \sigma_e^2 ZZ'
\]

\[
= \sigma_e^2 \begin{bmatrix} I \otimes \frac{1}{2} \\
\frac{1}{2} \otimes I \\
\end{bmatrix} \begin{bmatrix} I \otimes \frac{1}{2} \\
\frac{1}{2} \otimes I \\
\end{bmatrix}'
\]

\[
= \sigma_e^2 \begin{bmatrix} I \\
\frac{1}{2} \otimes I \\
\end{bmatrix}'
\]

\[
= \begin{bmatrix} \sigma_e^2 & \sigma_e^2 \\
\sigma_e^2 & \sigma_e^2
\end{bmatrix}
\]

= Block Diagonal with blocks

\[
\begin{bmatrix}
\sigma_e^2 & 0 \\
0 & \sigma_e^2
\end{bmatrix}
\]

\[
\begin{bmatrix}
\sigma_e^2 & 0 \\
0 & \sigma_e^2
\end{bmatrix}
\]
\[ \text{Var}(\varepsilon) = R = \sigma_e^2 I. \]

\[ \text{Var}(x) = \sigma_e^2 I \otimes \begin{pmatrix} 1 & 1' \\ 1 & 2 \end{pmatrix} + \sigma_e^2 I \]

= Block Diagonal with blocks

\[
\begin{bmatrix}
\sigma_e^2 + \sigma_e^2 & \sigma_e^2 \\
\sigma_e^2 & \sigma_e^2 + \sigma_e^2
\end{bmatrix}
\]
Conceptual Picture of the Experiment
Thus, the covariance between two observations from the same litter is $\sigma^2_\epsilon$ and the correlation is

$$\frac{\sigma^2_\epsilon}{\sigma^2_\epsilon + \sigma^2_e}.$$

This is also easy to compute from the non-matrix expression of the model.

\[\forall i,j \quad \text{Var}(y_{ijk}) = \text{Var}(M + a_i + B_j + y_{ij} + l_{ik} + e_{ijk}) = \text{Var}(l_{ik} + e_{ijk}) = \sigma^2_\epsilon + \sigma^2_e.\]
\[
\text{Cov}(Y_{i1k}, Y_{i2k}) = \text{Cov}(M + \alpha_1 + \beta_1 X_{i1} + \delta_{i1} + \epsilon_{i1k}, M + \alpha_2 + \beta_2 X_{i2} + \delta_{i2k} + \epsilon_{i2k}) \\
= \text{Cov}(\delta_{i1k} + \epsilon_{i1k}, \delta_{i2k} + \epsilon_{i2k}) \\
= \text{Cov}(\delta_{i1k}, \delta_{i2k}) + \text{Cov}(\delta_{i1k}, \epsilon_{i2k}) \\
+ \text{Cov}(\epsilon_{i1k}, \delta_{i2k}) + \text{Cov}(\epsilon_{i1k}, \epsilon_{i2k}) \\
= \text{Cov}(\delta_{i1k}, \delta_{i2k}) + 0 + 0 + 0 \\
= \text{Var}(\delta_{i1k}) = \sigma_{\delta}^2.
\]

\[
\text{Cor}(Y_{i1k}, Y_{i2k}) = \frac{\text{Cov}(Y_{i1k}, Y_{i2k})}{\sqrt{\text{Var}(Y_{i1k}) \text{Var}(Y_{i2k})}} = \frac{\sigma_{\delta}^2}{\sigma_{\delta}^2 + \sigma_{\epsilon}^2}.
\]