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.
Which Design to Use?

Consider a CRD if
1. There is equal interest in all treatment comparisons.
2. There is no natural way to group similar experimental units together in blocks.
3. No logistical constraints make complete randomization impractical.

Consider a RCBD if
1. There is equal interest in all treatment comparisons.
2. There are potentially important sources of variation that can be controlled by blocking.
3. No logistical constraints make complete randomization within blocks impractical.

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

How would you design the microarray portion of the experiment?

- Suppose the researcher in the split-plot experiment is willing to use 48 two-color microarray slides to measure gene expression in plants from the field.
- The researcher is primarily interested in understanding how gene expression changes in response to fertilizer rate within each genotype.

One Possible Design

Use a loop design to compare split-plot experimental units assigned adjacent fertilizer amounts within each whole-plot experimental unit.

Consider the same loop designs for blocks 2, 3, and 4 perhaps reversing loop directions for two of the four blocks.
Split-plot design structures don’t necessarily involve plots in the usual sense.

• Suppose a total of 8 pigs are assigned to two diets (A and B) using a completely randomized design with 4 pigs per diet.
• After 4 weeks on the assigned diet, each pig is sacrificed and RNA samples are taken from the stomach and small intestine of each pig.
• Affymetrix GeneChips are used to measure gene expression in each RNA sample with one GeneChip for each of the samples.

How would you design the microarray portion of the experiment?

• Suppose the researcher in this split-plot experiment can afford to use 8 two-color microarray slides to measure gene expression in the RNA samples.
• The researcher is primarily interested in understanding how diets affect gene expression within each organ type.

Note that the levels of the factor diet are randomly assigned to experimental units. That is not the case for the levels of the factor organ type, but we will analyze it and refer to it just as we would for an experimental factors whose levels are randomly assigned.
An Real Experiment Using Affymetrix GeneChips®

Barley Genotype

<table>
<thead>
<tr>
<th></th>
<th>Mia6</th>
<th>Mia13</th>
<th>Mia1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bgh 5874</td>
<td>Incompatible</td>
<td>Compatible</td>
<td>Incompatible</td>
</tr>
<tr>
<td>Bgh K1</td>
<td>Compatible</td>
<td>Incompatible</td>
<td>Incompatible</td>
</tr>
</tbody>
</table>


Experimental Design

This is a split-split-plot experimental design.

- Whole-plot experimental units are groups of three flats (trays containing plants).
- Split-plot experimental units are flats themselves.
- Split-split-plot experimental units are rows of seedlings within flats.

This is a split-split-plot experimental design.

- Whole-plot treatment factor is isolate with levels 5874 and K1.
- Split-plot treatment factor is genotype with levels Mia1, Mia6, and Mia13.
- Split-split-plot treatment factor is hours after inoculation with levels 0, 8, 16, 20, 24, and 32.