Statistical Design

Principles, Recommendations, and Opinions

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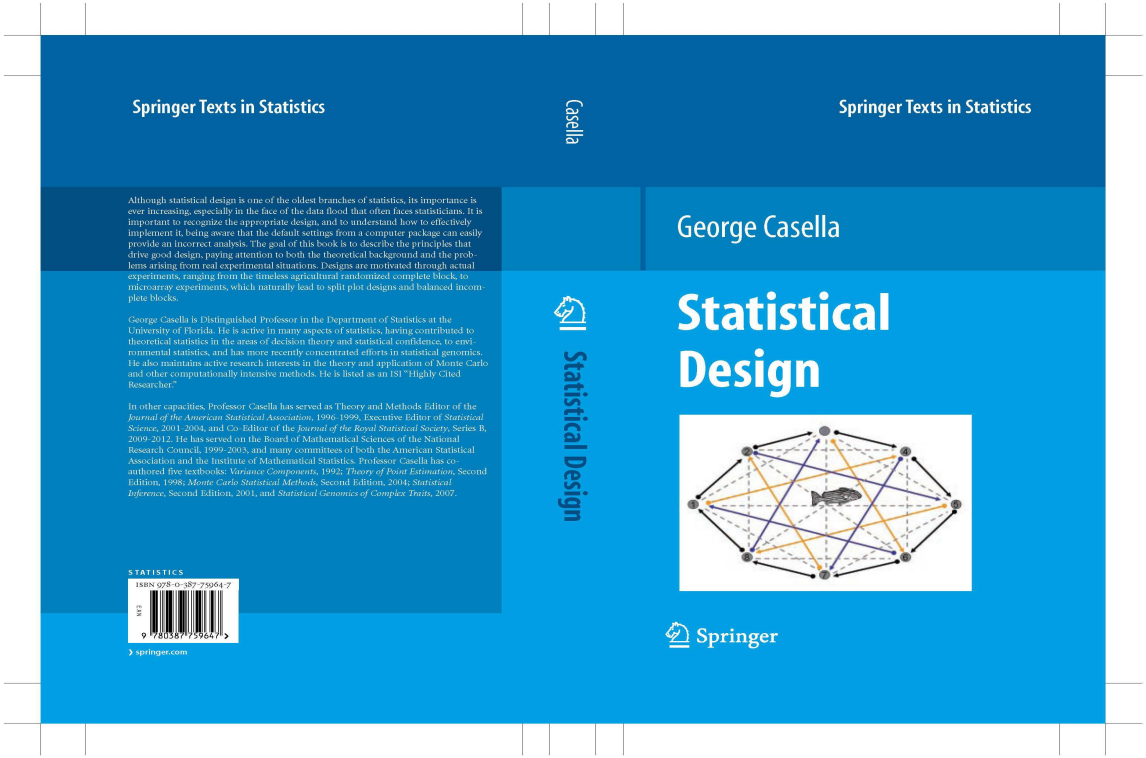
Based on

- Statistical Design, 2008, Springer-Verlag
- Data and R programs for the course available at http://www.stat.ufl.edu/ casella/StatDesign



And so it was ... borne in upon me that very often, when the most elaborate statistical refinements possible could increase the precision by only a few percent, yet a different design involving little or no additional experimental labour might increase the precision two-fold, or five-fold or even more..

R. A. Fisher

The Place of the Design of Experiments in the Logic of Scientific Inference, 1962

The Chapters

- Basics
- Completely Randomized Designs
- Blocking - Complete Designs
- Blocking with Fixed Blocks
- Split Plots
- Confounding - Incomplete Designs

Chapter 1: Basics

- Our concern is design, not analysis
- Good designs should result in a straightforward analysis
- Results presented in an anova framework,
  - Because the anova is the best way to think about data and plan designs.
  - Fisher (1934) first called the anova

    "a convenient method of arranging the arithmetic

- We first review "basics"

A Oneway Model

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}, \quad i = 1, \ldots, t; \quad j = 1, \ldots, r,$$

- Example:

Dry weight, in grams, of *Geranium* 'Dilys', subject to three fertilizer treatments.

Fertilizer		
A	B	C
1.02	1.00	.99
.79	1.21	1.36
1.00	1.22	1.17
.59	.96	1.22
.97	.79	1.12

- Oneway anova with

μ = true overall dry weight

τ_i = true change in dry weight due to fertilizer i

y_{ij} = observed yield of plant j in treatment i

ε_{ij} = unobserved error

Oneway Model Properties

- The model

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}, \quad i = 1, \ldots, t; \quad j = 1, \ldots, r,$$

  - is *overparameterized*
  - is *nonidentifiable*

- Identifiability restriction $\sum_i \tau_i = 0$.
- For example,

$$\mathrm{E}\,\bar{Y}_{i\cdot} = \frac{1}{r}\mathrm{E}\left(\sum_j \mu + \tau_i + \varepsilon_{ij}\right) = \mu + \tau_i,$$

$$\mathrm{E}\,\bar{Y} = \frac{1}{rt}\mathrm{E}\left(\sum_{ij} \mu + \tau_i + \varepsilon_{ij}\right) = \mu + \bar{\tau},$$

  - $\mu + \tau_i$ and $\mu + \bar{\tau}$ have unbiased estimators

Experimental Unit (EU)

- Perhaps the most important concept in statistical design
- The *experimental unit* is the unit (subject, plant, pot, animal) which is randomly assigned to a treatment.
- The experimental unit *defines the unit to be replicated to increase degrees of freedom.*
  - Fertilizer is applied to the pots. Plants are not the EU.
  - Different food placed in tanks containing the fish. Fish are not the EU
  - RNA is applied to a microarray. The EU is the subject.

Some Principles

- The experimental unit must be "randomly assigned".
  - One batch of fertilizer applied to five pots $\Rightarrow$ one EU
  - Food placed directly in the fish's mouth must be prepared independently for each fish

- A *sampling unit* is the object that is measured in an experiment. It may be different from the experimental unit.
- *Replication* is the repetition of the experimental situation by replicating the experimental unit.

  ▷ Experimental Unit=Pot

  ▷ Sampling Unit =Plant

Replication

- The anova table for the Fish Tanks is

Source	df	Mean Square	F Ratio
Diets	2	MS(Diet)	MS(Diet)/MS(Tank)
Tanks (in Diets)	9	MS(Tank)	
Fish (in Tanks)	60	MS(Fish)	

- F test on diets has low df.

- Replicating the fish is *subsampling* or *pseudo-replication*
- This is an example of a *nested* design
- Test tanks using $\frac{\text{MS(Tank)}}{\text{MS(Fish)}}$, typically not of interest

Know the Denominator

- Key principle
- For Tests or Intervals
  - Increase # of Pots (Plants don't help)
  - Increase # of Tanks (Fish don't help)
- That is, for a given number of plants (fish)
  - Maximize the number of pots(tanks)

Variance and Covariance

- In the model

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}, \quad i = 1, \ldots, t; \quad j = 1, \ldots, r,$$

with

$$\mathrm{E}(\varepsilon_{ij}) = 0, \qquad \mathrm{Var}(\varepsilon_{ij}) = \sigma^2.$$

- Can estimate all $\tau_i - \bar{\tau}$ and σ^2

$$\mathrm{E}\left(\bar{Y}_{i\cdot} - \bar{\bar{Y}}\right) = \tau_i - \bar{\tau}.$$

$$\mathrm{Var}\left(\bar{Y}_{i\cdot} - \bar{\bar{Y}}\right) = \frac{\sigma^2}{r}\left(1 - \frac{1}{t}\right)$$

My Favorite Formula

$$\mathrm{Var}(Y) = \mathrm{Var}[\mathrm{E}(Y|X)] + \mathrm{E}[\mathrm{Var}(Y|X)]$$

- Anova Decomposition
  - $\mathrm{SS}(\mathsf{Total}) = \mathrm{SS}(\mathsf{Trt}) + \mathrm{SS}(\mathsf{Within\ Trts})$
  - $\sum_{i=1}^{t}\sum_{j=1}^{r}(y_{ij} - \overline{\overline{y}})^2 = \sum_{i=1}^{t} r(\overline{y}_{i\cdot} - \overline{\overline{y}})^2 + \sum_{i=1}^{t}\sum_{j=1}^{r}(y_{ij} - \overline{y}_{i\cdot})^2.$
- Rao-Blackwell
  - $\mathrm{Var}(Y) \geq \mathrm{Var}[\mathrm{E}(Y|X)]$

Orthogonal and Uncorrelated

A Oneway Model

$$Y_{ij} = \theta_i + \varepsilon_{ij}, \quad i = 1, \ldots, t; \quad j = 1, \ldots, r_i,$$

$\sum_{i=1}^t a_i\theta_i$	$\sum_i a_i = 0$	Contrast
$\sum_{i=1}^t a_i\theta_i$ and $\sum_{i=1}^t b_i\theta_i$	$\sum_{i=1}^t a_i b_i = 0$	Orthogonal Contrasts
$\sum_{i=1}^t a_i\bar{y}_i$ and $\sum_{i=1}^t b_i\bar{y}_i$	$\sum_{i=1}^t a_i b_i = 0$	Orthogonal Contrasts
$\sum_{i=1}^t a_i\bar{y}_i$ and $\sum_{i=1}^t b_i\bar{y}_i$	$\sum_{i=1}^t a_i b_i/r_i = 0$	Uncorrelated Contrasts

- Do we want orthogonal or uncorrelated?

Rehabilitation Time

- $Y =$ rehabilitation time from knee surgery
- Group $=$ prior physical fitness
- 24 men, aged $18 - 30$

	Physical Condition			
	Poor Condition	Below Average	Above Average	Excellent Condition
	42	29	28	26
	⋮	⋮	⋮	⋮
	42	31	33	22
r	5	8	7	4

Which Contrasts?

	Physical Condition			
	Poor Condition	Below Average	Above Average	Excellent Condition
	42	29	28	26
	⋮	⋮	⋮	⋮
	42	31	33	22
r	5	8	7	4

μ_1	μ_2	μ_3	μ_4
1	-1/3	-1/3	-1/3
0	1	-1/2	-1/2
0	0	1	-1

Orthogonal

Does Not Partition Treatment SS

μ_1	μ_2	μ_3	μ_4
1	-8/19	-7/19	-4/19
0	1	-4/11	-7/11
0	0	1	-1

Uncorrelated

Partitions Treatment SS

But useless for inference

Randomization, Layouts and Designs

Example: Problematic Inference

- Forestry Experiment: Five varieties of Pine
- Four years of Greenhouse Experiments
- Variety B recommended as Best
  - Evidence Overwhelming
  - 10 lumber companies planted the recommended variety on half of their replacement acreage
  - 8 of the companies complained that variety B pine trees were only 75% as tall as "an old standby variety".
- What Happened?

Possible Explanations

(1) This all happened by chance.

(2) Trees were not randomly assigned in the greenhouse, and variety B received optimal conditions

(3) Experiment was properly done, but not representative.

- Randomization cannot do much about (1) or (3)
  - This is a Block $\times$ Treatment interaction
- Proper randomization should guard against (2).

Desirable Outcomes from Randomization

- Elimination of systematic bias.
  - Gradients of light or temperature,
  - Dye-bias in microarray experiments
  - Interviewer bias in surveys
- Obtaining a representative sample.
- Accounting for extraneous (unknown) confounding variables.

...the uncontrolled causes which may influence the result are always strictly innumerable

R. A. Fisher

The Design of Experiments, Section II.9

Treatment Design

- There are two parts to a design
- Experiment Design - Later
- Treatment Design
  - How the levels of treatments are arranged
  - Typically *crossed* or *nested*
  - Can be either *complete* or *incomplete*

Treatment Design

Crossed

A											
1			2			3			4		
B			B			B			B		
1	2	3	1	2	3	1	2	3	1	2	3
x	x	x	x	x	x	x	x	x	x	x	x

Nested

A											
1			2			3			4		
B			B			B			B		
1	2	3	4	5	6	7	8	9	10	11	12
x	x	x	x	x	x	x	x	x	x	x	x

This Confuses Students

- This "looks like" a oneway anova on treatments.

Treatment															
1					2					3					
Subject					Subject					Subject					
1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	
x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	

- But this is an RCB

	Subject				
	1	2	3	4	5
1	x	x	x	x	x
Treatment 2	x	x	x	x	x
3	x	x	x	x	x

- Random Factor $\Rightarrow$ Correlation
- Bring Correlation to the Top

The Treatment Design Tells us

- How to count degrees of freedom
- How to calculate sums of square
- How to calculate least squares estimates
  - We need more information to form F-ratios
  - That is the Experiment Design.

Experiment Design

- How EUs are randomized to treatments
- How the data are actually collected
  - The error structure is a consequence
  - Tells how to form F-ratios

Choices in a Twoway Crossed Design

- Possible anovas corresponding to
  - *(a)* complete randomization
  - *(b)* restriction of randomization of one factor
  - *(c)* restriction of randomization of both factors

	Choices		
	F-ratio		
Source	(a)	(b)	(c)
A	$\frac{\text{MS(A)}}{\text{MS(Within)}}$	$\frac{\text{MS(A)}}{\text{MS(Within)}}$	$\frac{\text{MS(A)}}{\text{MS(A} \times \text{B)}}$
B	$\frac{\text{MS(B)}}{\text{MS(Within)}}$	$\frac{\text{MS(B)}}{\text{MS(A} \times \text{B)}}$	$\frac{\text{MS(B)}}{\text{MS(A} \times \text{B)}}$
A $\times$ B	$\frac{\text{MS(A} \times \text{B)}}{\text{MS(Within)}}$	$\frac{\text{MS(A} \times \text{B)}}{\text{MS(Within)}}$	$\frac{\text{MS(A} \times \text{B)}}{\text{MS(Within)}}$
	CRD	A Random	B Random

Choices in a Twoway Crossed Design

- Possible field layouts corresponding to

(a) complete randomization - CRD

(b) restriction of randomization of one factor - RCB

(c) restriction of randomization of both factors - Strip Plot

(a)

A1B1	A2B1	A1B3
A1B2	A3B3	A3B2
A3B1	A2B2	A2B3

(b)

A2B1	A3B2	A1B3
A1B1	A2B2	A3B3
A3B1	A1B2	A2B3

(c)

A1B1	A1B2	A1B3
A3B1	A3B2	A3B3
A2B1	A2B2	A2B3

Replication: True and Technical

- True replication $\Rightarrow$ EU is replicated
- Technical replication $\Rightarrow$ EU is subsampled.

Example: Microarray Experiment

- RNA was harvested from two wild-type human cell lines
- They were grown unirradiated (U) or irradiated (I)
- Cell lines and irradiated state are crossed treatments.
  - RNA samples split: independent hybridizations A and B
  - Eight hybridizations

	U		I	
Sample 1	A	B	A	B
Sample 2	A	B	A	B

Which One?

1.

Treatment	
U	I
x	x
x	x
x	x
x	x

Anova

Source	df
Treatments(U/I)	1
Within	6
Total	7

2.

	Treatment	
	U	I
	x	x
Line 1	x	x
	x	x
Line 2	x	x

Anova

Source	df
Blocks(Lines)	1
Treatments(U/I)	1
B $\times$ T	1
Subsampling	4
Total	7

Pooling and Pooling

Example: Effect of shipping and storage on avocados

- Three shipping methods (increasingly expensive)
- Two storage methods (also increasingly expensive)
- Also Shipments (which act as blocks)
  - Four crates of avocados/each Trt combination
  - An RCB

		Shipment 1		Shipment 2		Shipment 3	
		Storage 1	Storage 2	Storage 1	Storage 2	Storage 1	Storage 2
Shipping Method	1	$x\ x$ $x\ x$	$x\ x$ $x\ x$	$x\ x$ $x\ x$	$x\ x$ $x\ x$	$x\ x$ $x\ x$	$x\ x$ $x\ x$
	2	$x\ x$ $x\ x$	$x\ x$ $x\ x$	$x\ x$ $x\ x$	$x\ x$ $x\ x$	$x\ x$ $x\ x$	$x\ x$ $x\ x$
	3	$x\ x$ $x\ x$	$x\ x$ $x\ x$	$x\ x$ $x\ x$	$x\ x$ $x\ x$	$x\ x$ $x\ x$	$x\ x$ $x\ x$

Two Anovas

Anova

Source	df
Blocks (Shipments)	2
Shipping Method	2
Storage	1
Shipping × Storage	2
Residual	64
Total	71

- Naive Analysis
- All Tests Against Residual
- Pooling Interaction and Within inflates α (anticonservative)
- The 54 df are wasted

Source	df	
Blocks(Shipments)	2	
Shipping	2	
Storage	1	
Shipping × Storage	2	
Residual	64	
B × Ship		4
B × Stor		2
B × Ship × Stor		4
Within		54
Total	71	

- Better Analysis
- Individual Tests?
- Pooling three interaction terms is conservative

...and Pooling EUs, such as RNA

- Changes the EU from the subject to the pool of subjects
  - The between subject variation, is reduced
  - The df are based on the number of pools, not subjects

$$\mathrm{Var}(\bar{Y}_{i..}) = \frac{1}{rp}\left(\sigma_B^2 + \frac{\sigma_W^2}{s}\right).$$

- r= # of replications
- p= # of pools
- s= # of subsamples

Example

1. Give randomization plans for the following two experiments:

(1) Treatment A, three varieties of alfalfa, is crossed with treatment B, three types of fertilizer. The response variable is dry weight.

(2) Blood pressure of human subjects is measured. Classification A, consisting of three age classes, is crossed with classification B, consisting of three weight classes.

Randomizations

- Possible Randomizations

(a)

A1B1	A2B1	A1B3
A1B2	A3B3	A3B2
A3B1	A2B2	A2B3

(b)

A2B1	A3B2	A1B3
A1B1	A2B2	A3B3
A3B1	A1B2	A2B3

(c)

A1B1	A1B2	A1B3
A3B1	A3B2	A3B3
A2B1	A2B2	A2B3

(1) Randomization Throughout. Choose a variety and a treatment at random, or choose a weight class and an age class at random, and take the measure.

(2) The Fertilizeris applied to a plot, and three levels of Variety are randomized. Or we choose and age class at random, and measure three people of different weights.

(3) Fertilizer is applied in one direction, and Varieties are planted in the other. This is problematic for the other experiment, as the treatments are not "applied".

Chapter 2: Completely Randomized Designs

If the idea looked lousy, I said it looked lousy. If it looked good, I said it looked good. Simple proposition.

Richard P. Feynman

Surely You're Joking, Mr. Feynman

Introduction

- CRDs have only fixed factors
- All tests against within error
- A model for the twoway CRD is

$$Y_{ijk} = \mu + \tau_i + \gamma_j + (\tau\gamma)_{ij} + \varepsilon_{ijk},$$
$$i = 1, \ldots, t; \quad j = 1, \ldots, g, \quad k = 1, \ldots, r$$

  - Y_{ijk} is the observed response
  - τ_i is one treatment effect
  - γ_j is the other treatment effect
  - $(\tau\gamma)_{ij}$ represents the interaction
  - ε_{ijk} is the error

CRD Assumptions

$$Y_{ijk} = \mu + \tau_i + \gamma_j + (\tau\gamma)_{ij} + \varepsilon_{ijk},$$
$$i = 1, \ldots, t; \quad j = 1, \ldots, g, \quad k = 1, \ldots, r$$

- $\varepsilon_{ijk} \sim \mathsf{N}(0, \sigma^2)$
- $\mathrm{Corr}(\varepsilon_{ijk}, \varepsilon_{i'j'k'}) = 0.$

- We can also also assume (for free)

$$\bar{\tau} = \bar{\gamma} = \overline{(\tau\gamma)} = 0,$$

  - Just redefines μ

CRD Assumptions

$$Y_{ijk} = \mu + \tau_i + \gamma_j + (\tau\gamma)_{ij} + \varepsilon_{ijk}$$

- For identifiability we need

$$(\bar{\tau\gamma})_{i\cdot} = (\bar{\tau\gamma})_{\cdot j} = 0, \text{ for all } i, j$$

- This is not free
- This is a redefinition of the parameters

$$\begin{aligned}
\tau_i' &= \tau_i + (\bar{\tau\gamma})_{i\cdot} \\
\gamma_j' &= \gamma_j + (\bar{\tau\gamma})_{\cdot j} \\
(\tau\gamma)_{ij}' &= (\tau\gamma)_{ij} - (\bar{\tau\gamma})_{i\cdot} - (\bar{\tau\gamma})_{\cdot j}.
\end{aligned}$$

- The average interaction effect does not go away
  - It relocates

Twoway Example

Example: The effect of sulphur and nitrogen on red clover

- Dry matter yields, grams/pot

Nitrogen	Sulphur 0	Sulphur 3	Sulphur 6	Sulphur 9
0	4.48	4.70	5.21	5.88
0	4.52	4.65	5.23	5.98
0	4.63	4.57	5.28	5.88
20	5.76	7.01	5.88	6.26
20	5.72	7.11	5.82	6.26
20	5.78	7.02	5.73	6.37

- Twoway CRD anova

Source	df	Sum Sq	Mean Sq	F	p
Sulphur	3	3.06	1.02	285.53	$< .00001$
Nitrogen	1	7.83	7.83	2185.63	$< .00001$
Sulphur $\times$ Nitrogen	3	3.76	1.25	349.78	$< .00001$
Within	16	0.057	0.0036		

Expected Mean Squares and F-tests

- Indicates the correct denominators for F-test
- Shows which replication controls sources of variation
- Helps us in setting up a better design.

Expected Mean Squares for twoway CRD anova

Source	df	EMS
Treatment T	$t-1$	$\sigma^2 + \frac{rg}{t-1}\sum_i \tau_i^2$
Treatment G	$g-1$	$\sigma^2 + \frac{rt}{g-1}\sum_j \gamma_i^2$
T $\times$ G	$(t-1)(g-1)$	$\sigma^2 + \frac{r}{(t-1)(g-1)}\sum_{ij}(\tau\gamma)_{ij}^2$
Within	$tg(r-1)$	σ^2

- $H_0 : \sum_i \tau_i^2 = 0$, etc.

Estimating Contrasts

Under the model

$$Y_{ijk} \sim N\left(\mu + \tau_i + \gamma_j + (\tau\gamma)_{ij}, \sigma^2\right), \quad \text{Cov}(Y_{ijk}, Y_{i'j'k'}) = 0,$$

- $\sum_i a_i \bar{Y}_{i\cdot\cdot} \sim N\left(\sum_i a_i \tau_i, \frac{\sigma^2}{rg}\sum_i a_i^2\right)$
- $\frac{\sum_i a_i \bar{Y}_{i\cdot\cdot} - \sum_i a_i \tau_i}{\sqrt{\frac{\hat{\sigma}^2}{rg}\sum_i a_i^2}} \sim t_{tg(r-1)}.$
  - With $\hat{\sigma}^2 = \text{MS}(\text{Within})/tg(r-1)$
- This follows from Cochran's Theorem

Cochran's Theorem

Theorem

- $\mathbf{Y} \sim N(0, \Sigma)$
- A_k, $k = 1, 2, \ldots, m$ satisfy $\sum_{k=1}^{m} A_k = A$
- $A\Sigma$ is idempotent

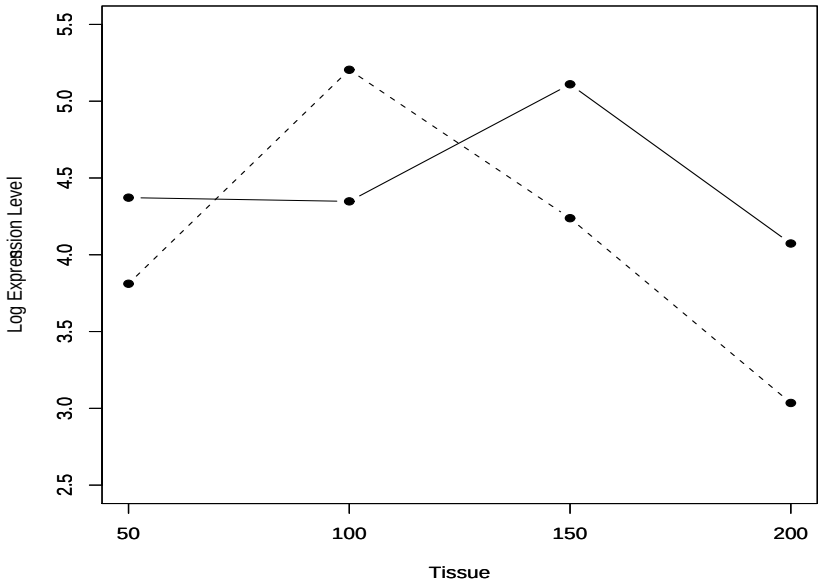
If $A_k\Sigma$ is idempotent for every k and $A_k\Sigma A_{k'} = 0, \quad k \neq k'$,

(1) $\mathbf{Y}'A_k\mathbf{Y} \sim \chi^2_{tr(A_k\Sigma)}$ for every k

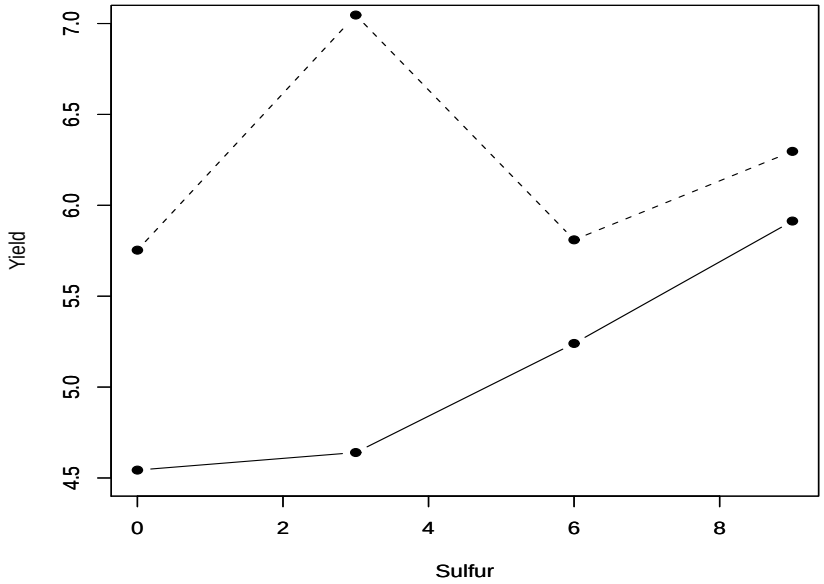
(2) $\mathbf{Y}'A_k\mathbf{Y}$ and $\mathbf{Y}'A_{k'}\mathbf{Y}$ are independent for $k \neq k'$

(3) $\mathbf{Y}'A\mathbf{Y} \sim \chi^2_{tr(A\Sigma)}$.

Interactions



Interaction plot
- fish tissue experiment-
Qualitative



Interaction plot
- red clover experiment-
Quantitative

Adjusting for Covariates

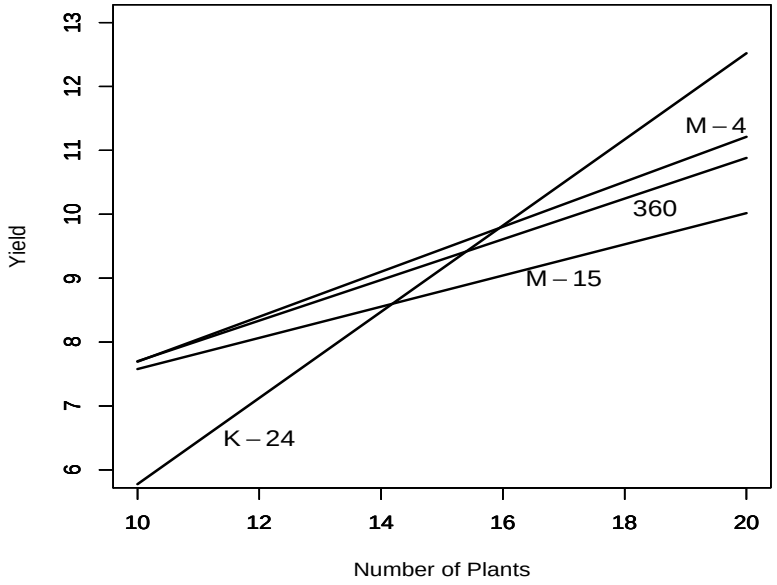
- A covariate is like a block, in that it removes variation.
- A covariate satisfies two conditions:
  - The covariate is related to the response, and can account for variation
  - The covariate is not related to the treatment. Important!

Response	Covariate
plant yield	density/plot of the plants
growth of laboratory rats	initial weight of the rats
florescence of a spot	spot size

Covariates

	Varieties							
	Cornell M-4		Robson 360		Ohio K-24		Ohio M-15	
Obs.	X	Y	X	Y	X	Y	X	Y
1	20	12.8	20	12.2	20	14.1	13	8.6
2	17	11.0	20	10.0	20	13.1	18	10.2
3	20	10.9	16	9.8	20	12.8	17	8.7
4	15	9.1	20	9.8	20	11.8	14	7.3
5	20	9.6	19	9.8	20	10.8	15	9.3
6	15	9.3	20	12.1	13	7.8	11	8.2

- Yields of varieties of corn
- Covariate = # plants/plot



- Regardless of the treatment positive relationship between yield and # plants/plot

Ancova Models

- Oneway anova model

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}, \quad i = 1, \ldots t, \quad j = 1, \ldots, r.$$

- An ancova model

$$Y_{ij} = \mu + \tau_i + \beta(x_{ij} - \bar{x}) + \varepsilon_{ij}, \quad i = 1, \ldots t, \quad j = 1, \ldots, r,$$

  - In each group, the slope is the same.
  - This assumption is both crucial and bothersome

Ancova - Testing Treatments

- The ancova hypotheses

$$H_0 : Y_{ij} = \mu + \beta(x_{ij} - \bar{x}) + \varepsilon_{ij} \text{ vs. } H_1 : Y_{ij} = \mu + \tau_i + \beta(x_{ij} - \bar{x}) + \varepsilon_{ij},$$

- Two anova tables for the corn data are

Covariate After Treatment

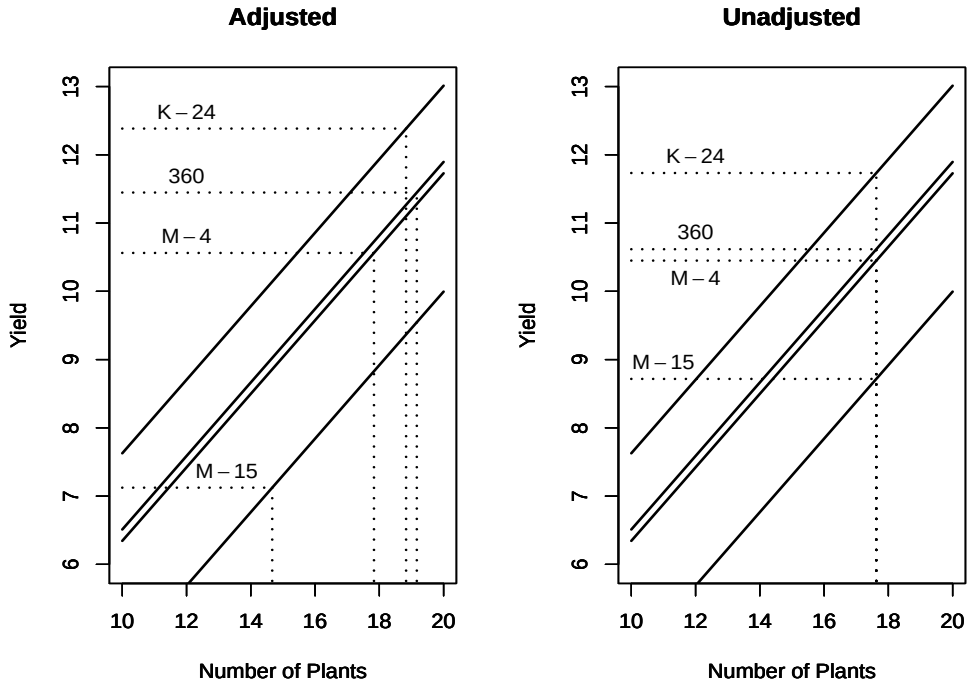
Source	df	SS	MS
Varieties	3	27.955	9.318
Within	20	46.765	2.338
Plants (after Varieties)	1	21.729	21.729
Residual	19	25.036	1.318

Treatment After Covariate

Source	df	SS	MS
Plants	1	43.916	43.916
Residual (from Regression)	22	30.804	1.400
Varieties (after Plants)	3	5.768	1.923
Residual	19	25.036	1.318

- $F = \dfrac{\text{Varieties (after Plants)}}{\text{Residual}}$

Adjusted Means



	Varieties			
	360	K-24	M-15	M-4
Unadjusted Mean	10.617	11.733	8.717	10.450
Std. Error	0.624	0.624	0.624	0.624
Adjusted Mean	11.447	12.384	7.124	10.562
Std. Error	0.496	0.486	0.563	0.469

- Anova adjusts to overall mean
- Ancova adjusts to covariate means
- Variances may be reduced

Variances May Be Reduced

- Average estimated variance

$$\mathrm{Var}\left((\widehat{\mu+\tau_i})-(\widehat{\mu+\tau_{i'}})\right)=\frac{2\hat{\sigma}^2}{r}\left(1+\frac{1}{(t-1)}\frac{\mathrm{SS}(\mathsf{Trt}_x)}{\mathrm{SS}(\mathsf{Within}_x)}\right),$$

- The variance $\downarrow$ as $\hat{\sigma}^2\downarrow$
  - The regression of Y on X improves

- The variance $\uparrow$ if X is related to the treatment
  - $\mathrm{SS}(\mathsf{Trt}_x)/\mathrm{SS}(\mathsf{Within}_x)\uparrow$

Example

- Fish microarray experiment
- Two treatments: Tissue Mass and presence or absence of hCG (hormone)
- Treatment design:

		Tissue Mass (mg)			
		50	100	150	200
hCG	Yes	x	x	x	x
	No	x	x	x	x

- Tissue Mass qualitative suggests polynomial contrasts.

Example

- A full set of orthogonal contrasts
- Contrasts can be generated in R with statements such as `contr.poly` or `contr.helmert`

Linear Tissue Mass (mg)

hCG	−3	−1	1	3
1	−3	−1	1	3
−1	3	1	−1	−3

Quadratic Tissue Mass (mg)

hCG	1	−1	−1	1
1	1	−1	−1	1
−1	−1	1	1	−1

Cubic Tissue Mass (mg)

hCG	−1	3	−3	1
1	−1	3	−3	1
−1	1	−3	3	−1

Example

- Anova Table

Source	df
Tissue Mass	3
Linear	1
Quadratic	1
Cubic	1
hCG	1
Tissue Mass × hCG	3
Linear × hCG	1
Quadratic × hCG	1
Cubic × hCG	1
Within	4
Total	11

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Tissue	3	1.67479	0.55826	0.7910	0.5589
hCG	1	0.43426	0.43426	0.6153	0.4767
Tissue:hCG	3	71.49048	0.49683	0.7039	0.5975
Residuals	4	2.823	19	0.70580	

Answers

(a) For the linear interaction we have

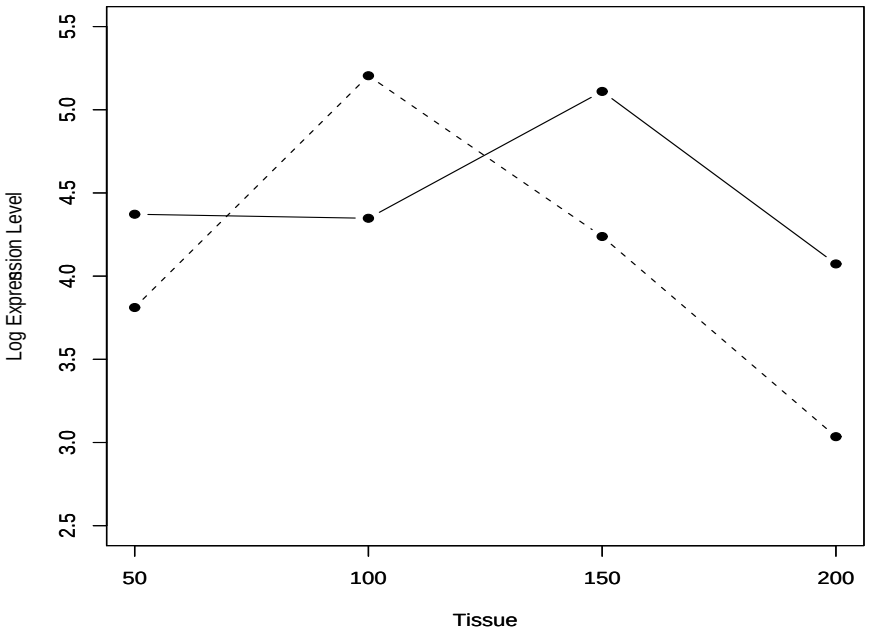
	Tissue			
hCG	-3	-1	1	3
	3	1	-1	3

	Lin	Quad	Cubic
SS	0.3333	0.4181	0.7391
F	0.4722	0.5924	1.0472
p	0.5298	0.4844	0.3640

- Not much happening

(b) For the main effect of tissue:

	Lin	Quad	Cubic
SS	0.1263	1.5482	0.0003
F	0.1789	2.1936	0.0004
p	0.6941	0.2127	0.9853



- Pretty Picture. Still not much happening.

Chapter 3: Randomized Complete Blocks

We shall need to judge of the magnitude of the differences introduced by testing our treatments upon the different plots by the discrepancies between the performances of the same treatment in different blocks.

R. A. Fisher

The Design of Experiments, Section 26

I thanked him for the explanation; now I understood it. I have to understand the world, you see.

Richard P. Feynman

Surely You're Joking, Mr. Feynman

Fixed or Random

- Blocks are typically treated as a random effect
- Clear instances where blocks are not random
- Covariance is the key to modeling
- Block Assumption $\Rightarrow$ Scope of Inference
  - Inference to Blocks in the model = Fixed Factor
  - Inference to Blocks beyond the model = Random Factor
- Covariance is the key to modeling
  - Yes, I know I repeated this!

Fixed or Random

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  - Yes, I know I repeated this!

Fixed and Random Blocks

A	C	B
10.1	8.4	6.3

B	C	A
6.9	9.4	10.8

C	A	B
9.0	9.8	5.3

A	C	B
10.5	9.2	6.2

- Three varieties of plants
- Four Blocks
- Random Blocks

Brand A

	T		
P	x	x	x
	x	x	x
	x	x	x
	x	x	x

Brand B

	T		
P	x	x	x
	x	x	x
	x	x	x
	x	x	x

Brand C

	T		
P	x	x	x
	x	x	x
	x	x	x
	x	x	x

- Six Treatment Combinations
- Three Brands (Blocks)
- Fixed Blocks

- In either case, correlation in the blocks

Put the covariance at the top - Revisited

- A city considers purchasing outside sculpture pieces
- 40 pieces of art considered, grouped into four categories
  - A total of 12 judges were available
  - Each judge rated each piece of art on a 7-point scale
- Here is the data layout

		Category			
		1	2	3	4
		Art	Art	Art	Art
		$1\ 2\ \cdots\ 10$	$11\ 12\ \cdots\ 20$	$21\ 22\ \cdots\ 30$	$31\ 32\ \cdots\ 40$
Judges	1	$x\ x\ \cdots\ x$	$x\ x\ \cdots\ x$	$x\ x\ \cdots\ x$	$x\ x\ \cdots\ x$
	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
	12	$x\ x\ \cdots\ x$	$x\ x\ \cdots\ x$	$x\ x\ \cdots\ x$	$x\ x\ \cdots\ x$

- What is the design?

Possible Anovas

		Category 1	2	3	4
		Art	Art	Art	Art
		1 2 ⋯ 10	11 12 ⋯ 20	21 22 ⋯ 30	31 32 ⋯ 40
Judges	1	$x\ x\ \cdots\ x$	$x\ x\ \cdots\ x$	$x\ x\ \cdots\ x$	$x\ x\ \cdots\ x$
	⋮	⋮	⋮	⋮	⋮
	12	$x\ x\ \cdots\ x$	$x\ x\ \cdots\ x$	$x\ x\ \cdots\ x$	$x\ x\ \cdots\ x$

- Original Analysis
- Fully Nested
- Covariance mishandled

Source	df
Cat	3
Art (in Cat)	36
Judges (in Art)	440
Total	479

- Recommended Analysis
- RCB
- Covariance correctly modelled

Source	df
Judges (Blocks)	11
Cat	3
Art (in Cat)	36
Cat × Judges	33
Art × Judges (in Cat)	396
Total	479

Definitions

- The blocks are called *complete* blocks if every treatment appears in every block,
- Classical model (no interaction?)

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}, \quad i = 1, \ldots, t, \quad j = 1, \ldots, b,$$

  - One observation for each treatment–block combination
  - No two observations taken under the same conditions.
  - A most efficient design

- Randomization: In each block, the treatments are run in a completely random manner

RCB with Interaction

$$Y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk},$$
$$i = 1, \ldots, t, \quad j = 1, \ldots, b, \quad k = 1, \ldots, r$$

- In the RCB the error comes from the variation of treatment contrasts across blocks, not from within a cell.

Source	df	SS	MS	F
Blocks	$b-1$	SS(Blocks)		
Trts	$t-1$	SS(Trt)	MS(Trt)	$F = \frac{\text{MS(Trt)}}{\text{MS(T} \times \text{B)}}$
T $\times$ B	$(b-1)(t-1)$	SS(T $\times$ B)	MS(T $\times$ B)	$F =$?
Subsampling	$bt(r-1)$	SS(Within)	MS(Within)	

- What is subsampling (Within) good for?

RCB with Interaction

- Extra samples typically subsamples of the EU
  - Test on treatments is exactly the same
  - Presence of the within doesn't matter
  - Waste of effort with respect to the test on treatments

Source	df	Sum Sq	Mean Sq	F	p
Block	3	3.982	1.327		
Variety	3	37.201	12.400	26.068	0.000
Variety $\times$ Block	9	4.281	0.476	1.880	0.092
Within	32	8.100	0.253		

- Variety tested by Variety $\times$ Block
- Three observations/cell doesn't help here
- We may be able to test the interaction

Purpose of Blocking

- Blocking serves many purposes
- Within a block there is homogeneity
  - Treatment comparisons are very precise
- Between blocks there is heterogeneity
  - Treatments compared across a variety of situations
- We want "significant" blocks

$$\mathrm{SS}(\mathsf{Total}) - \mathrm{SS}(\mathsf{Treatments}) = \mathrm{SS}(\mathsf{Blocks}) + \mathrm{SS}(\mathsf{T} \times \mathsf{B}).$$

Microarray Example

- Microarray Stem Cell experiment
- Effect of G-CSF on White blood cell production
  - The dataset `StemCell` contains data for 250 genes

		Genes			
Subject	Trt	AFFX-BioB-5-at	AFFX-BioB-M-at	AFFX-BioB-3-at	AFFX-BioC-5-at
1	Post	961	1734.3	825.7	2746.8
1	Pre	734.8	1239.7	607.3	2425
2	Post	1737.2	2926.7	1602.2	5256.6
2	Pre	755.5	1215.3	670.9	2306.3
3	Post	777.4	1597.8	750.3	2723.9
3	Pre	791.1	1349.7	711.2	2134.3
4	Post	1022.5	1761.7	871.8	2958.9
4	Pre	706.6	1145.8	596.1	2189
5	Post	754.9	1374.1	637.2	2334.4
5	Pre	809.8	1262.9	629.1	2100.7

- RCB for each gene
- Subject = Blocks

Means and Variances

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}, \quad i = 1, \ldots, t, \quad j = 1, \ldots, b,$$

- $\varepsilon_{ij} \sim$ iid $\mathsf{N}(0, \sigma_\varepsilon^2)$
- $\beta_1, \ldots, \beta_b$, are iid $\mathsf{N}(0, \sigma_\beta^2)$ and are independent of ε_{ij}
  - The mean and variance of Y_{ij}, conditional on the β_js:

$$\mathrm{E}(Y_{ij}) = \mu + \tau_i + \beta_j, \quad \mathsf{Var}\,(Y_{ij}) = \sigma_\varepsilon^2.$$

  - The unconditional mean and variance of Y_{ij} are

$$\mathrm{E}Y_{ij} = \mu + \tau_i, \quad \mathsf{Var}\,Y_{ij} = \sigma_\beta^2 + \sigma_\varepsilon^2.$$

Correlation

- Conditional on blocks

$$\mathrm{Cov}(Y_{ij}, Y_{i'j'}|\beta_j, \beta_{j'}) = \mathrm{Cov}(\varepsilon_{ij}, \varepsilon_{i'j'}) = 0$$

- Unconditionally

$$\mathrm{Cov}(Y_{ij}, Y_{i'j}) = \mathrm{Cov}(\beta_j+\varepsilon_{ij}, \beta_j+\varepsilon_{i'j}) = \mathrm{Cov}(\beta_j, \beta_j) = \sigma^2_\beta$$

  - Positive covariance in the blocks
  - A consequence of the model

- Unconditional (Intraclass) Correlation

$$\mathrm{Corr}(Y_{ij}, Y_{i'j}) = \frac{\mathsf{Cov}(Y_{ij}, Y_{i'j})}{\sqrt{(\mathsf{Var}\, Y_{ij})(\mathsf{Var}\, Y_{i'j})}} = \frac{\sigma^2_\beta}{\sigma^2_\beta + \sigma^2_\varepsilon},$$

Expected Squares and F-tests

- EMS: one observation per treatment-block combination
- Cochran's Theorem applies - equicorrelation

Source	df	EMS
Blocks	$b-1$	$\sigma^2_\varepsilon + t\sigma^2_\beta$
Treatments	$t-1$	$\sigma^2_\varepsilon + \frac{b}{t-1}\sum_i [\tau_i]^2$
TxB	$(t-1)(b-1)$	σ^2_ε

- Test $H_0 : \tau_i = 0$ for all i with

$$\frac{\text{MS(Trts)}}{\text{MS(T} \times \text{B)}} \sim F_{t-1,(b-1)(t-1)}.$$

Estimating Contrasts

- Use Least Squares Estimates
- Estimate $\sum_i a_i\tau_i$ with $\sum_i a_i\hat{\tau}_i$ where

$$\mathrm{E}\left(\sum_i a_i\hat{\tau}_i\right) = \sum_i a_i\tau_i \text{ and } \mathrm{Var}\left(\sum_i a_i\hat{\tau}_i\right) = \frac{\sigma_\varepsilon^2}{b}\sum_i a_i^2.$$

- Inference is Straightforward
- Note

$$\sigma_\varepsilon^2 = \text{“Residual”} = \mathsf{T} \times \mathsf{B}$$

even if we don't model it!

Modelling the Interaction

- Recall True vs. Technical Replication

(1) Technical Replication: if RNA from the *same* subject is used in two different microarrays.

▷ True replication would have RNA from different subjects on each microarray.

(2) Technical Replication: In an block, if fertilizer is applied to a subplot with 5 plants from the same line, clone, etc., then the 5 plants are a technical replication.

▷ True Replication: In a block, if the treatment is applied to the plot, and we have independent replicates.

▷ True Replication: In a block, if the treatment is applied to the plant, and we have independent replicates.

▷ Conditional Independence

Modelling the Correlation

- True vs. Technical affects the correlation
- For $k \neq k'$, but in the same block,

$$\mathrm{Corr}(\varepsilon_{ijk}, \varepsilon_{i'jk'}) = \begin{cases} \rho_\varepsilon & \text{for technical replication} \\ 0 & \text{for true replication} \end{cases}$$

- A similar distinction is made by Gates (1995)

Models

- Many extensions of "no-interaction" model
- We use Model II (Hocking 1973, 1985)

$$Y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk},$$
$$i = 1, \ldots, t, \quad j = 1, \ldots, b, \quad k = 1, \ldots, r,$$

  - $\varepsilon_{ijk} \sim \quad \mathsf{N}(0, \sigma^2)$
  - $\mathrm{Corr}(\varepsilon_{ijk}, \varepsilon_{i'jk'}) = \rho_\varepsilon$
  - $(\tau\beta)_{11}, \ldots, (\tau\beta)_{tb}, \sim \quad \mathsf{N}(0, \sigma^2_{\tau\beta})$
  - $\mathrm{Corr}((\tau\beta)_{ij}, (\tau\beta)_{i'j}) = 0$
  - $\beta_1, \ldots, \beta_b$, are *iid* $\mathsf{N}(0, \sigma^2_\beta)$, independent

Tests

- If $\rho_\varepsilon \neq 0$,
  - Can test treatments with T $\times$ B
  - Cannot test T $\times$ B using Within
- Can only test T $\times$ B using Within if $\rho_\varepsilon = 0$
- Also have Intraclass Correlation

$$\text{Corr}(Y_{ijk}, Y_{ijk'}) = \frac{\sigma^2_\beta + \sigma^2_{\tau\beta} + \rho_\varepsilon \sigma^2_\varepsilon}{\sigma^2_\beta + \sigma^2_{\tau\beta} + \sigma^2_\varepsilon} \quad \text{[inside T} \times \text{B]}$$

$$\text{Corr}(Y_{ijk}, Y_{i'jk'}) = \frac{\sigma^2_\beta}{\sigma^2_\beta + \sigma^2_{\tau\beta} + \sigma^2_\varepsilon} \quad \text{[inside B]}$$

EMS - RCB with Interaction

Source	df	EMS
Blocks	$b-1$	$\sigma^2_\varepsilon[1+(r-1)\rho_\varepsilon]+r\sigma^2_{\tau\beta}[1+(t-1)\rho_{\tau\beta}]+rt\sigma^2_\beta$
Treatments	$t-1$	$\sigma^2_\varepsilon[1+(r-1)\rho_\varepsilon]+r\sigma^2_{\tau\beta}+\frac{rt}{t-1}\sum_i(\tau_i-\bar{\tau})^2$
TxB	$(t-1)(b-1)$	$\sigma^2_\varepsilon[1+(r-1)\rho_\varepsilon]+r\sigma^2_{\tau\beta}$
Within	$bt(r-1)$	$(1-\rho_\varepsilon)\sigma^2_\varepsilon$

- There is always a test for treatments
- Cannot Test Interaction Unless
  - $\rho_\varepsilon = 0$ True Replication

Model II

- The standard Model II has all ε_{ijk} independent
  - This implies that $\rho_{\varepsilon} = 0$
  - But this cannot always be assumed
- Note that $\mathrm{Cov}(\bar{Y}_{ij\cdot}, \bar{Y}_{i'j\cdot}) \neq 0$
  - Even if we assume $\rho_{\varepsilon} = 0$
  - We always have intraclass correlation

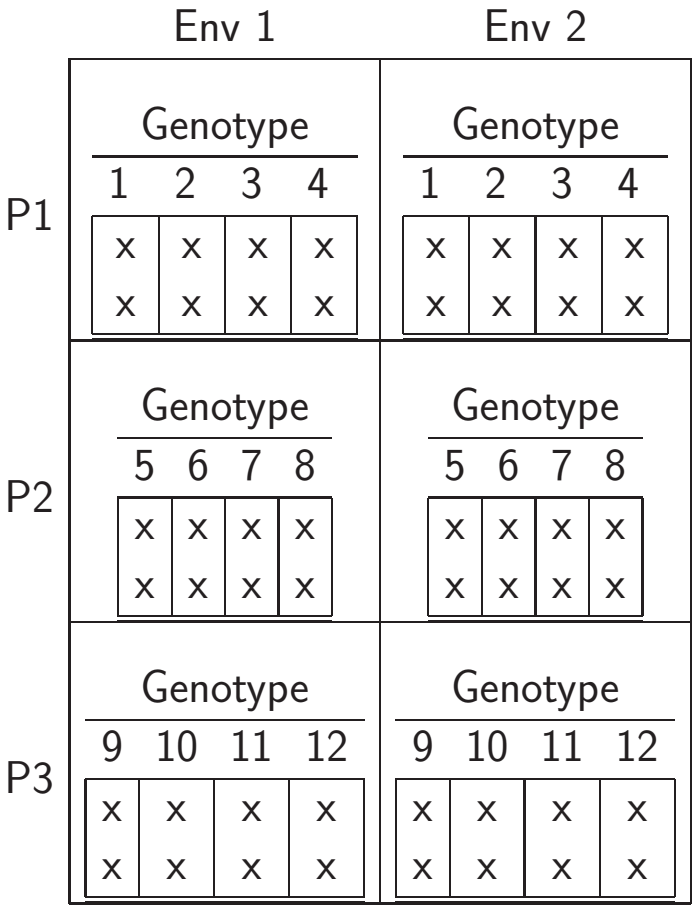
RCB - Treatment Design

- Three parental lines of *Persea americana*, or avocado
- Interest in treatment differences and env. interactions
  - Trees cloned, planted in two locations
  - Clones (Parents) are crossed with environment

Source	df
Env	1
Parent	2
E x P	2
Genotype (in P)	9
G x E (in P)	9
Within	24
Total	47

- This is an RCB
- Trt. Design = Nested
- Tests?

RCB - Treatment Design



Source	df
Env	1
Parent	2
E x P	2
Genotype (in P)	9
G x E (in P)	9
Within	24
Total	47

- $\frac{\text{Parent}}{\text{E x P}}$
- $\frac{\text{Genotype (in P)}}{\text{G x E (in P)}}$
- $\frac{\text{G x E (in P)}}{\text{Within}}$

Variations on a Theme

- Some Variations of Blocking
  - Replicating the Experiment
  - Crossed Blocks
  - Latin Squares

Replicating the Experiment

- Replication by repeating the entire experiment
- Often good reasons to do so
  - Agricultural - Replicate over Years
  - Microarray - Replicate over Labs
- Surprisingly, the valid tests are not what you may expect!

Blocks Nested in Reps

Replications

1	2	·····	r

	Block		
Trt	1	···	b
1	x	···	x
⋮	⋮	⋮	⋮
t	x	···	x

	Block		
Trt	1	···	b
1	x	···	x
⋮	⋮	⋮	⋮
t	x	···	x

·····

	Block		
Trt	1	···	b
1	x	···	x
⋮	⋮	⋮	⋮
t	x	···	x

Source	df	SS	MS
Location	2	3.119	1.559
Blocks(in Locations)	12	17.017	1.4181
Variety	4	4.516	1.129
Variety × Location	8	1.702	0.213
Variety × Block (in Location)	48	5.843	0.122

- Tests?
- Can we use the 48 df?

Blocks Nested in Reps

Source	df	SS	MS
Location	2	3.119	1.559
Blocks(in Locations)	12	17.017	1.4181
Variety	4	4.516	1.129
Variety × Location	8	1.702	0.213
Variety × Block (in Location)	48	5.843	0.122

- Can't use 48 df
- Without Assumptions
- Like $\sigma^2_{\tau R} = 0$

- $F = \dfrac{\text{Trt}}{\text{Trt} \times \text{Rep}}$

Expected Mean Squares

Source	df	EMS
Replications	r-1	$\sigma^2_\varepsilon + \sigma^2_{\tau\beta} + t\sigma^2_\beta + b\sigma^2_{\tau R} + bt\sigma^2_R$
Blocks (in Reps)	r(b-1)	$\sigma^2_\varepsilon + \sigma^2_{\tau\beta} + t\sigma^2_\beta$
Treatments	t-1	$\sigma^2_\varepsilon + \sigma^2_{\tau\beta} + b\sigma^2_{\tau R} + \frac{rb}{t-1}\sum_i \tau_i^2$
Trt × Rep	(t-1)(r-1)	$\sigma^2_\varepsilon + \sigma^2_{\tau\beta} + b\sigma^2_{\tau R}$
Trt × Block (in Rep)	r(t-1)(b-1)	$\sigma^2_\varepsilon + \sigma^2_{\tau\beta}$
Total	btr-1	

Similar result if blocks crossed with reps

Crossed Blocks

Blocks B

Blocks C	1	2	⋯	b
1	T: 1 2 ⋯ t / x x ⋯ x / . . ⋯ . / x x ⋯ x	T: 1 2 ⋯ t / x x ⋯ x / . . ⋯ . / x x ⋯ x	⋯	T: 1 2 ⋯ t / x x ⋯ x / . . ⋯ . / x x ⋯ x
2	T: 1 2 ⋯ t / x x ⋯ x / . . ⋯ . / x x ⋯ x	T: 1 2 ⋯ t / x x ⋯ x / . . ⋯ . / x x ⋯ x	⋯	T: 1 2 ⋯ t / x x ⋯ x / . . ⋯ . / x x ⋯ x
⋮	⋮	⋮	⋯	⋮
g	T: 1 2 ⋯ t / x x ⋯ x / . . ⋯ . / x x ⋯ x	T: 1 2 ⋯ t / x x ⋯ x / . . ⋯ . / x x ⋯ x	⋯	T: 1 2 ⋯ t / x x ⋯ x / . . ⋯ . / x x ⋯ x

- B and C are blocks
- T is randomized on the intersection of B and C
- Can account for two gradients
- "Full Factorial" Latin Square

Crossed Blocks - The Bad News

Source	df	Expected mean squares EMS
Blocks B	b-1	$\sigma^2_\varepsilon + \sigma^2_{\beta\tau\gamma} + t\sigma^2_{\beta\gamma} + g\sigma^2_{\tau\beta} + tg\sigma^2_\beta$
Blocks C	g-1	$\sigma^2_\varepsilon + \sigma^2_{\beta\tau\gamma} + t\sigma^2_{\beta\gamma} + r\sigma^2_{\tau\gamma} + tr\sigma^2_\gamma$
T	t-1	$\sigma^2_\varepsilon + \sigma^2_{\beta\tau\gamma} + g\sigma^2_{\tau\beta} + r\sigma^2_{\tau\gamma} + \frac{rg}{t-1}\sum_i \tau_i^2$
Blocks B × T	(b-1)(t-1)	$\sigma^2_\varepsilon + \sigma^2_{\beta\tau\gamma} + g\sigma^2_{\tau\beta}$
Blocks C × T	(g-1)(t-1)	$\sigma^2_\varepsilon + \sigma^2_{\beta\tau\gamma} + r\sigma^2_{\tau\gamma}$
Blocks B × Blocks C	(b-1)(g-1)	$\sigma^2_\varepsilon + \sigma^2_{\beta\tau\gamma} + t\sigma^2_{\beta\gamma}$
Blocks B × Blocks C × T	(b-1)(g-1)(t-1)	$\sigma^2_\varepsilon + \sigma^2_{\beta\tau\gamma}$
Total	bgt-1	

- No Direct test on treatments
  - Can assume either $\sigma^2_{\tau\gamma} = 0$ or $\sigma^2_{\tau\beta} = 0$
  - Satterthwaite approximation
- Can test T × Block interaction

Latin Squares

- Crossed Blocks $\Rightarrow$ Latin Squares
- Each Intersection has only one treatment
  - Controls Two Gradients
  - Each row contains exactly one level of each treatment
  - Each column contains exactly one level of each treatment
- Now we see the assumptions needed for inference

Latin Square Setup

Blocks B East-West

Blocks C North-South	1	2	3	4
1	T_3	T_1	T_2	T_4
2	T_1	T_2	T_4	T_3
3	T_2	T_4	T_3	T_1
4	T_4	T_3	T_1	T_2

Source	df	SS	MS	F	p-value
Row	3	9.427	3.142		
Column	3	245.912	81.971		
Treatment	3	23.417	7.806	1.953	.223
Residuals	6	23.984	3.997		
Total	15	302.74			

- t Treatments $\Rightarrow$ t Rows and t Columns - A Square!
- The "Residuals" are a soup of interactions

Interpretation

Source	df	SS	MS	F	p-value
Row	3	9.427	3.142		
Column	3	245.912	81.971		
Treatment	3	23.417	7.806	1.953	.223
Residuals	6	23.984	3.997		
Total	15	302.74			

Source	df	SS	MS
Row	3	9.427	3.142
Column	3	245.912	81.971
Row $\times$ Column	9	47.401	5.267
Total	15	302.74	

- SS(Treatments) is from the Row $\times$ Column effect
- Essential that there is no Row $\times$ Column effect
  - The residual should only measure experimental error
  - Otherwise test is conservative (?)

Latin Square Model

$$Y_{i_{jk}} = \mu + \tau_i + \beta_j + \gamma_k + \varepsilon_{jk}$$

- Index set a bit involved
- Only one i for each jk

		Blocks B East-West 1	2	3	4
Blocks	1	$\tau_3 + \beta_1 + \gamma_1$	$\tau_1 + \beta_2 + \gamma_1$	$\tau_2 + \beta_3 + \gamma_1$	$\tau_4 + \beta_4 + \gamma_1$
C	2	$\tau_1 + \beta_1 + \gamma_2$	$\tau_2 + \beta_2 + \gamma_2$	$\tau_4 + \beta_3 + \gamma_2$	$\tau_3 + \beta_4 + \gamma_2$
North-South	3	$\tau_2 + \beta_1 + \gamma_3$	$\tau_4 + \beta_2 + \gamma_3$	$\tau_3 + \beta_3 + \gamma_3$	$\tau_1 + \beta_4 + \gamma_3$
	4	$\tau_4 + \beta_1 + \gamma_4$	$\tau_3 + \beta_2 + \gamma_4$	$\tau_1 + \beta_3 + \gamma_4$	$\tau_2 + \beta_4 + \gamma_4$

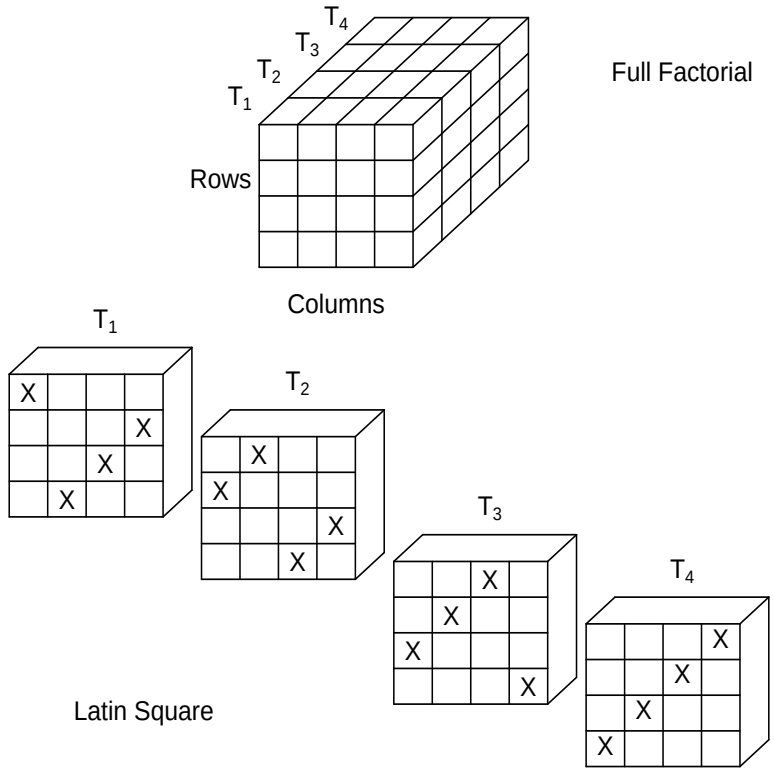
- Notice that Block effects sum to zero (balanced)
- Treatment contrasts free of block effects

Latin Square Contrasts

$$\begin{aligned} \mathrm{E}\left(\sum_i a_i \bar{Y}_i\right) &= \sum_i a_i \tau_i \\ \mathrm{Var}\left(\sum_i a_i \bar{Y}_i\right) &= \frac{\sigma_\varepsilon^2}{t} \sum_i a_i^2 \end{aligned}$$

- σ_ε^2 is the residual term
- Estimated with MS(Residual) with $(t-2)(t-1)$ df
  - Latin Squares can be replicated to increase residual df
- Variation: *Latin Rectangle*.
  - Rows crossed with Reps, Columns nested
  - Similar analysis

Some Observations



- An RCB if Rows or Columns ignored
- Here, only need $1/4$ of the observations of full factorial

More Observations

Rows \ Columns	1	2	3	4	5
1	A	B	C	D	E
2	B	C	D	E	A
3	C	D	E	A	B
4	D	E	A	B	C
5	E	A	B	C	D

- Standard Square
- ABCDE = first row and column
- Can always use cyclic construction

- Randomization: Choose at random from all squares
- 12 3×3 squares, 576 4×4 squares, $161,280$ 5×5 squares
  - Listing becomes problematic
  - In practice: Randomly permute rows and columns of standard square

RCD - Some Final Notes

- Cochran's Theorem
  - Works here - Covariance Matrix is Equicorrelated
  - Details in Text
- Mixed Model Estimation
  - Prediction of Block Effects
- Other Models
  - Scheffé
- Variance Components
  - REML

An Example

Revisiting the Alfalfa Experiment

- Four varieties of alfalfa, RCB with four blocks
- Response variable was yield, in tons of dry hay per acre
- For each Variety $\times$ Block cell there were three subsamples

- Anova table

Source	df	Sum Sq	Mean Sq	F	p
Block	3	3.982	1.327		
Variety	3	37.201	12.400	26.068	.000
Variety $\times$ Block	9	4.281	0.476	1.880	.092
Within	32	8.100	0.253		

An Example

Revisiting the Alfalfa Experiment - 2

Source	df	Sum Sq	Mean Sq	F	p
Block	3	3.982	1.327		
Variety	3	37.201	12.400	26.068	.000
Variety $\times$ Block	9	4.281	0.476	1.880	.092
Within	32	8.100	0.253		

- Testing the interaction term is often of lesser interest.
- The existence of interaction is an academic question
- By their very nature, we cannot control blocks

Other Designs

Revisiting the Alfalfa Experiment - 3

Eight Blocks

Source	df
Block	7
Variety	3
V × B	21
Within	16

Twelve Blocks

Source	df
Block	11
Variety	3
V × B	33
Within	0

- The 8 block anova is unbalanced in the cells.
- Better design uses 12 blocks; most df for the Variety test.

- The eight block design could be preferred if there concern about interaction.
- The 4 block experiment is a waste of effort.

Chapter 4:
Interlude: Assessing the Effects of Blocking

The first principle is that you must not fool yourself...
Richard P. Feynman
Surely You're Joking, Mr. Feynman

Introduction

- Random Blocks $\Rightarrow$ The levels selected are a random sample
- Puzzling: We almost never actually take a random sample

- In a sense the concept of a random factor is a fallacy
  - And is often difficult to explain to students

- Important implication: Blocking induces a *correlation*
- This correlation can be modelled directly.

- So model a factor according to what it really is!

Fixed or Random?

- Five varieties of tomato plant
- Different levels of light
- Blocks = Area of Greenhouse

• Fixed

- Eighteen subjects
- Measure memorization
- Trt = level of distraction
- Blocks = Subjects

• Random

- Five elementary schools
- Three methods of teaching
- Measure scores on pre-post test
- Blocks = Schools

• Fixed

Model and Inference

- Blocks are fixed or random $\Rightarrow$ can affect some calculations
- The scope of the inference will be affected
  - Inference can only go to the blocks used

- Model

$$Y_{ij} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ij}, \quad i = 1, \ldots, t, \quad j = 1, \ldots, b,$$

  - $\varepsilon_{ij} \sim \mathsf{N}(0, \sigma^2)$
  - $\mathrm{Corr}(\varepsilon_{ij}, \varepsilon_{i'j'}) = \begin{cases} \rho & \text{if } j = j', i \neq i' \\ 0 & \text{otherwise} \end{cases}$

Expected Mean Squares

EMS for RCB anova with fixed blocks and replication

Source	df	EMS
Blocks	$b-1$	$\sigma^2\left[1+(r-1)\rho_\varepsilon+r(t-1)\rho_B\right]+\frac{rt}{b-1}\sum_j \beta_j^2$
Treatments	$t-1$	$\sigma^2[1-r\rho_B+(r-1)\rho_\varepsilon]+\frac{br}{t-1}\sum_i \tau_i^2$
TxB	$(t-1)(b-1)$	$\sigma^2[1-r\rho_B+(r-1)\rho_\varepsilon]+\frac{r}{(b-1)(t-1)}\sum_{ij}(\tau\beta)_{ij}^2$
Within	$bt(r-1)$	$(1-\rho_\varepsilon)\sigma^2$

- Can always test treatments
- Interaction test problematic even with true replication

Remarks

- There are implicit identities in random blocks model
- Cannot happen with Fixed Blocks
  - Can test $H_0 : (\tau\beta)_{ij} = 0$ if $\rho_\varepsilon = 0$
- In the end, fixed or random blocks is really not our choice
  - Reality of the experiment
  - Scope of inference
- The correlation not necessarily restricted to be positive, as in random blocking.

Chapter 5: Split Plot Designs

"How absurdly simple!", I cried.
"Quite so!", said he, a little nettled. "Every problem becomes very childish when once it is explained to you."

Dr. Watson and Sherlock Holmes
The Adventure of the Dancing Men

Introduction

- The workhorse of statistical design
- If the only tool you own is a hammer, then everything in the world looks like a nail
  - From now on, you will see the split plot in almost every design that you encounter
- A *split plot design* (*or split unit design*) is one in which there is more than one type of experimental unit.

A Split Plot Example

- Study of dietary composition on health
  - Four diets were randomly assigned to 12 subjects
  - Blood pressure was measured morning and evening

	Diet			
	1	2	3	4
	Subject 1 2 3	Subject 4 5 6	Subject 7 8 9	Subject 10 11 12
Morning	$x\ x\ x$	$x\ x\ x$	$x\ x\ x$	$x\ x\ x$
Evening	$x\ x\ x$	$x\ x\ x$	$x\ x\ x$	$x\ x\ x$

- There are 12 subjects (EU) but there are 24 numbers
- The experimental unit is split

Split Plot Anova

Source	df
Diets	3
Subjects (in Diets)	8
Time	1
Time × Diet	3
Time × Subjects (in Diets)	8
Total	23

- Whole Plots above the line
- Split Plots below the line

- The split plot design is an experiment design
- An implied correlation structure
- The whole plots act as blocks for the split plot treatment
- Comparisons "below the line" have greater precision.
  - Put the important stuff here! (If possible)

CRD on the Whole Plots

The split plot model, with whole plot treatments in a CRD,

$$Y_{ijk} = \mu + \tau_i + \varepsilon_{ij} + \gamma_k + (\tau\gamma)_{ik} + \delta_{ijk}$$

- $\varepsilon_{ij} =$ whole plot error, $\varepsilon_{ij} \overset{\text{iid}}{\sim} N(0, \sigma^2_\varepsilon)$
- $\delta_{ijk} =$ split plot error, $\overset{\text{iid}}{\sim} N(0, \sigma^2_\delta)$, independent of ε_{ij}.

Data Layout for Split Plot Design

	T			
	1	2	⋯	t

		Rep						Rep					
		1	2	⋯	r	⋯		1	2	⋯	r
G	1	x	x	⋯	x	⋯	G	x	x	⋯	x
	2	x	x		x			x	x		x
	⋮	⋮	⋮		⋮			⋮	⋮		⋮
	g	x	x		x			x	x		x

Model Consequences

- The whole plot analysis is based only on the $\bar{y}_{ij}$
  - Can be done in ignorance of what goes on below the line
  - The $\bar{y}_{ij}$ are independent
- There is correlation below the line

$$\mathrm{Corr}(Y_{ijk}, Y_{ijk'}) = \frac{\sigma_\varepsilon^2}{\sigma_\varepsilon^2 + \sigma_\delta^2}.$$

  - Equicorrelation
- The split plot error is a pooled interaction term

EMS and F-tests

- Estimation and testing is, for the most part, straightforward

EMS for a split plot design, whole plots in a CRD

Source	df	EMS
Whole Plot Trt	t-1	$\sigma^2_\delta + g\sigma^2_\varepsilon + \frac{rg}{t-1}\sum_i \tau_i^2$
Replication (in Whole Plots)	t(r-1)	$\sigma^2_\delta + g\sigma^2_\varepsilon$
Split Plot Trt	g-1	$\sigma^2_\delta + \frac{rt}{g-1}\sum_k \gamma_k^2$
Split Plot Trt $\times$ Whole Plot Trt	(g-1)(t-1)	$\sigma^2_\delta + \frac{r}{(g-1)(t-1)}\sum_{ik}(\tau\gamma)^2_{ik}$
Split Plot Trt $\times$ Replication (in Whole Plots)	t(g-1)(r-1)	σ^2_δ
Total	grt-1	

- Here the tests are clear
- Cochran's Theorem Applies

Estimating Contrasts

There are four types of contrasts to consider:

- Whole Plot Means: $\sum_i a_i \tau_i$, where $\sum_i a_i = 0$
- Split Plot Means: $\sum_k a_k \gamma_k$, where $\sum_k a_k = 0$
- Interaction Means, Same Level of Whole Plot: $\sum_k a_k (\tau\gamma)_{ik}$, where $\sum_k a_k = 0$
- Interaction Means, Different Whole Plot Level: $\sum_{ik} a_{ik} (\tau\gamma)_{ik}$, where $\sum_{ik} a_{ik} = 0$

Estimating Contrasts

Whole Plot Means $\mathrm{Var}(\sum_i a_i \bar{Y}_i) = \left(\frac{\sigma^2_\varepsilon}{r} + \frac{\sigma^2_\delta}{rg}\right) \sum_i a_i^2$

Split Plot Means $\mathrm{Var}\left(\sum_k a_k \bar{Y}_k\right) = \frac{\sigma^2_\delta}{tr} \sum_k a_k^2$

Interaction Means, Same Whole Plot $\mathrm{Var}\left(\sum_k a_k \bar{Y}_{ik}\right) = \frac{\sigma^2_\delta}{r} \sum_k a_k^2$

Interaction Means, Different Whole Plot $\mathrm{Var}\left(\sum_{ik} a_{ik} \bar{Y}_{ik}\right) = \frac{\sigma^2_\delta}{r} \sum_{ik} a_{ik}^2 + \frac{\sigma^2_\varepsilon}{r} \sum_i \left(\sum_k a_{ik}\right)^2$

- $\sum_k a_{ik} = 0$ if SP comparisons are balanced

Dietary Split Plot Example

Source	df	SS	MS	F	p-value
Diet	3	1873.46	624.49	85.16	$< .0001$
Subject(in Diet)	8	58.667	7.333		
Time	1	1190.04	1190.04	73.6108	$< .0001$
Diet $\times$ Time	3	53.13	17.71	1.095	0.405
Split Plot Error	8	129.33	16.17		

		Time	
		AM	PM
Diet	1	121.67	133.33
	2	121.33	139.00
	3	112.67	129.00
	4	139.67	150.33

Same WP

	AM	PM
1	1	-1
2	0	0
3	0	0
4	0	0

Within WP

	AM	PM
1	1	-1
2	1	-1
3	0	0
4	0	0

Between WP

	AM	PM
1	1	0
2	-1	0
3	0	0
4	0	0

Interaction

	AM	PM
1	1	-1
2	-1	1
3	0	0
4	0	0

Dietary Split Plot Example

Same WP

	AM	PM
1	1	-1
2	0	0
3	0	0
4	0	0

Case (3)

Within WP

	AM	PM
1	1	-1
2	1	-1
3	0	0
4	0	0

Case (3)

Between WP

	AM	PM
1	1	0
2	-1	0
3	0	0
4	0	0

Case (4)

Interaction

	AM	PM
1	1	-1
2	-1	1
3	0	0
4	0	0

Case (3)

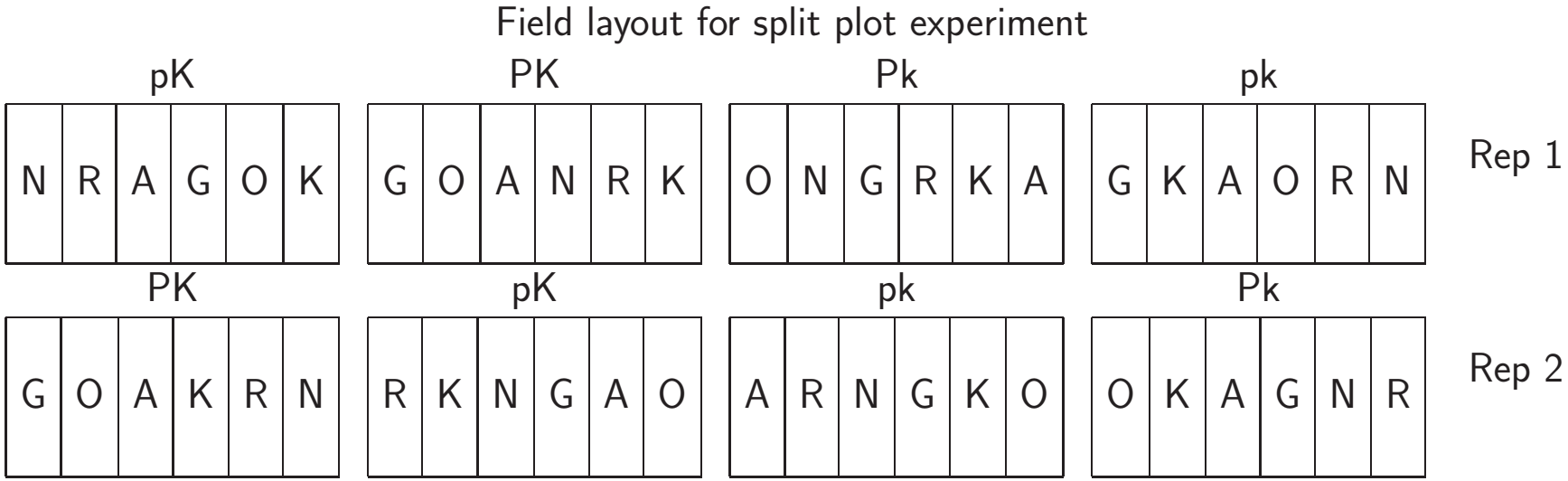
- First two contrasts: SP Trt. within levels of the WP Trt.
  - We have $\sum_k a_{ik} = 0$, so we are in Case (3)
- Third contrast: Cell means from different whole plots
  - We have $\sum_k a_{ik} \neq 0$, so we are in Case (4)
- Fourth contrast is an interaction of cell means
  - We have $\sum_k a_{ik} = 0$, so we are back in Case (3)

RCB on the Whole Plots

- We have seen SP designs with a CRD on the WP Trts.
- There is no restriction to the whole plot treatment design.
  - A more popular setup is to have the whole plots in an RCB
  - This does not change computations and inference too much
  - But does have an interesting effect on the SP error terms

Variety Split Plot

- A classic split plot done at the Cornell Experiment Station
- Compare alfalfa varieties response to fertilizer treatments
  - Whole plots are in an RCB
  - Split plots are completely randomized in whole plots



Model and Distribution Assumptions

A model is

$$Y_{ijk} = \mu + \tau_i + \beta_j + \varepsilon_{ij} + \gamma_k + (\tau\gamma)_{ik} + (\beta\gamma)_{jk} + \delta_{ijk},$$

where $i = 1, \ldots, t$, $j = 1, \ldots, r$, $k = 1, \ldots, g$.

- $\beta_j =$ whole plot block $\overset{\text{iid}}{\sim} N(0, \sigma^2_\beta)$
- $\varepsilon_{ij} =$ whole plot error, $\varepsilon_{ij} \overset{\text{iid}}{\sim} N(0, \sigma^2_\varepsilon)$
- $(\beta\gamma)_{ik} =$ block-treatment interaction $\overset{\text{iid}}{\sim} N(0, \sigma^2_{\beta\gamma})$
- $\delta_{ijk} =$ split plot error, $\overset{\text{iid}}{\sim} N(0, \sigma^2_\delta)$

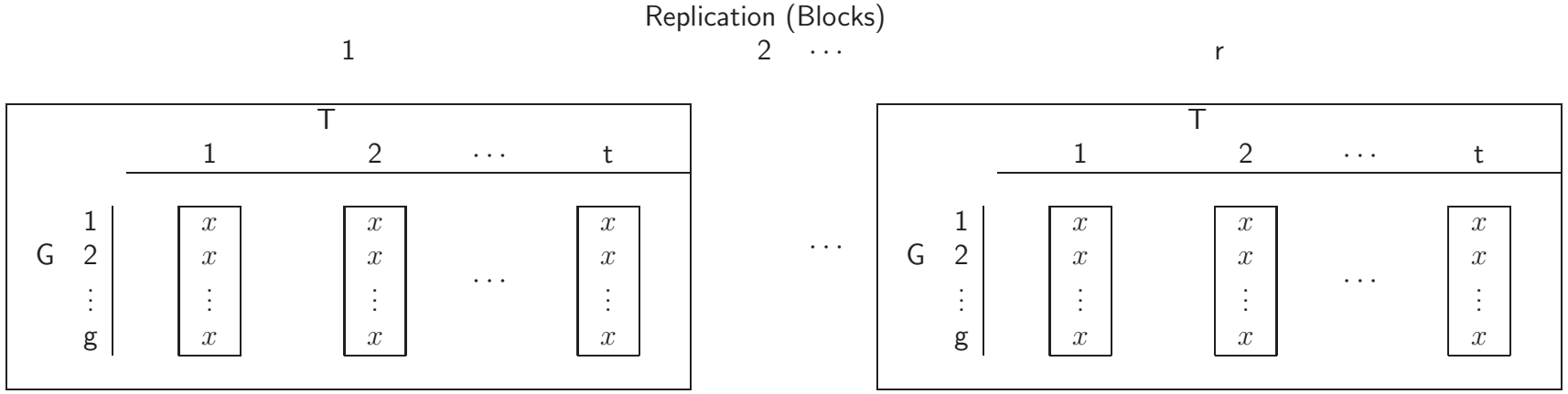
- We assume that all error terms are independent

Model Consequences

- Block structure $\Rightarrow$ two new random effects.
- This results in a more complicated split plot error term
- Note that the ε_{ij} is the Block $\times$ Treatment interaction
- The correlation structure a bit more complicated
  - If $j \neq j'$, $\mathrm{Corr}(Y_{ijk}, Y_{i'j'k'}) = 0$ (blocks independent)
  - Otherwise

	Same WP	Different WP
Same SP	-	$\sigma^2_\beta + \sigma^2_{\beta\gamma}$
Different SP	$\sigma^2_\beta + \sigma^2_\varepsilon$	σ^2_β

Data Layout and Anova



Source	df	SS	MS	F	p-value
Rep	1	6.961	6.961		
Trt	3	14.775	4.925	19.811	0.018
Trt × Rep	3	0.746	0.2486		
Variety	5	2.071	0.414	$\frac{.414}{0.369} = 1.122$	.451
Trt × Variety	15	1.526	0.102	$\frac{.102}{.104} = .977$	0.518
Variety × Rep	5	1.849	0.369		
Trt × Variety × Rep	15	1.562	0.104		

- Note the two error terms below the line

EMS and Errors

EMS for RCB Split Plot

Source	df	EMS
Blocks	b-1	$\sigma^2_\delta + g\sigma^2_\varepsilon + t\sigma^2_{\beta\gamma} + gt\sigma^2_\beta$
Whole Plot Trt	t-1	$\sigma^2_\delta + g\sigma^2_\varepsilon + \frac{bg}{t-1}\sum_i \tau_i^2$
Blocks × WP Trts	(b-1)(t-1)	$\sigma^2_\delta + g\sigma^2_\varepsilon$
Split Plot Trt	g-1	$\sigma^2_\delta + t\sigma^2_{\beta\gamma} + \frac{bt}{g-1}\sum_k \gamma_k^2$
Split Plot Trt × Whole Plot Trt	(g-1)(t-1)	$\sigma^2_\delta + \frac{b}{(g-1)(t-1)}\sum_{ik}(\tau\gamma)^2_{ik}$
Blocks × SP Trt	(b-1)(g-1)	$\sigma^2_\delta + t\sigma^2_{\beta\gamma}$
Blocks × SP Trt × WP Trts	(b-1)(g-1)(t-1)	σ^2_δ
Total	bgt-1	

- The presence of $\sigma^2_{\beta\gamma} \Rightarrow$ two error terms below the line.
- Pooling these errors assumes $\sigma^2_{\beta\gamma} = 0$
  - Reasonable if whole plots randomly assigned to split plots
  - Pooling into "Split Plot Error" is conservative

Estimating Contrasts

Whole Plot Means $\mathrm{Var}(\sum_i a_i \bar{Y}_i) = \frac{\sigma^2_\delta + g\sigma^2_\varepsilon}{bg} \sum_i a_i^2$

Split Plot Means $\mathrm{Var}\left(\sum_k a_k \bar{Y}_k\right) = \frac{\sigma^2_\delta + t\sigma^2_{\beta\gamma}}{bt} \sum_k a_k^2$

Interaction Means, Same Whole Plot $\mathrm{Var}\left(\sum_k a_k \bar{Y}_{ik}\right) = \frac{\sigma^2_\delta + \sigma^2_{\beta\gamma}}{b} \sum_k a_k^2$

Interaction Means, Different Whole Plot $\mathrm{Var}\left(\sum_{ik} a_{ik} \bar{Y}_{ik}\right) = \frac{\sigma^2_\delta}{b} \sum_{ik} a_{ik}^2 + \frac{\sigma^2_{\beta\gamma}}{b} \sum_k \left(\sum_i a_{ik}\right)^2 + \frac{\sigma^2_\varepsilon}{b} \sum_i \left(\sum_k a_{ik}\right)^2$

- No obvious estimate of $\sigma^2_\delta + \sigma^2_{\beta\gamma}$
- Last expression can be nasty

One Last Split Plot

- `Affymetrix` oligonucleotide microarrays
  - Single-dye system
- The experimental unit is the RNA
  - On the chip are the genes
  - Here we split the EU - get expression level of all genes
- The genes are a split plot treatment
- The WP treatments (chips) can have different designs
  - CRD, RCB, or something else.

Microarray Split Plot

- With a oneway CRD for the whole plots, a model is

$$y_{ijk} = \mu + T_i + A_{ij} + G_k + (GT)_{ik} + \varepsilon_{ijk},$$

- The anova is

Source	df
Treatments	t-1
Whole Plot Error	t(r-1)
Genes	g-1
Gene $\times$ Treatment	(t-1)(g-1)
Split Plot Error	t(g-1)(r-1)

- Microarrays must be replicated - otherwise no tests!
  - Interest in G and $G \times T$
  - This test is at the split-plot level, and is more precise.

Splitting Twice

- SP design $\Rightarrow$ SP Trt is randomized to levels of the WP Trt
- We can continue, creating a *split split plot* design.
  - The splits should be dictated by the physical constraints, and perhaps the desire for greater accuracy in the measurement of a particular treatment.
  - We'll be less formal here, looking at some examples.
  - Of course, we could split more than twice and, for example, have a split split split plot design.
  - We will look the CRD. RCB split split is nastier.

Ozone Chamber Split Split

- Test the effect of ozone gas on plants
- Two environmental chambers for each of four ozone levels
  - Six varieties of plants were placed in each chamber
  - Data from two locations/plant - root (R) and top (T)

Ozone Level

1								.....	4							
Chamber									Chamber							
1				2					7				8			
		Loc.				Loc.				Loc.				Loc.		
		R	T			R	T			R	T			R	T	
	1	x	x		1	x	x	.....		1	x	x		1	x	x
	2	x	x		2	x	x			2	x	x		2	x	x
Var.	3	x	x	Var.	3	x	x		Var.	3	x	x	Var.	3	x	x
	4	x	x		4	x	x			4	x	x		4	x	x
	5	x	x		5	x	x			5	x	x		5	x	x
	6	x	x		6	x	x			6	x	x		6	x	x

Ozone Chamber Split Split

Source	df
Ozone	3
Whole Plot Error (Chambers in Ozone)	4
Variety	5
V × O	15
Split Plot Error (V × C in O)	20
Location	1
L × V	5
L × O	3
L × V × O	15
Split Split Plot Error (L × C in V × O)	24
Total	95

- The treatments (L, V, O) are crossed.
- The random factor Chambers is nested in WP
- WP and SP errors are the same as before

- WP error comes from the replication of the WP treatments
- SP errors come from the respective interactions
- SSP error is the L × random factor C, nested in V × O.

Randomization Patterns

- Three crossed factors, A, B, and C, each at three levels
- CRD, SP, and SSP
- Possible randomization of the first nine observations.

		A									
		1			2			3			
		B			B			B			
		1	2	3	1	2	3	1	2	3	
C	1		3			5		4			CRD
	2	8	1						7	9	
	3		2							6	

C	1				4	9	7				A=Whole Plots
	2				1	6	5				B × C = Split Plots
	3				8	2	3				

C	1	4	7	1							A=Whole Plots
	2	6	9	3							B = Split Plots
	3	5	8	2							C = Split Split Plots

Variations on a Theme

- We briefly look at three variations of the split plot design

- The strip plot design
  - Reflects a specific type of randomization
- The crossover design
  - A useful variation of the SP
  - More common in experiments using human subjects.
- The repeated measures design
  - Brings in a new error structure

Strip Plot Designs

- Effect of potassium and phosphorus on yield of sugarcane.
- Use farm-scale equipment to apply the chemicals

Field Layout and Yield

	Block I			Block II			Block III		
	K3	K1	K2	K1	K3	K2	K2	K1	K3
P1	56	32	49	38	62	50	63	54	68
P2	67	54	58	52	72	64	54	44	51

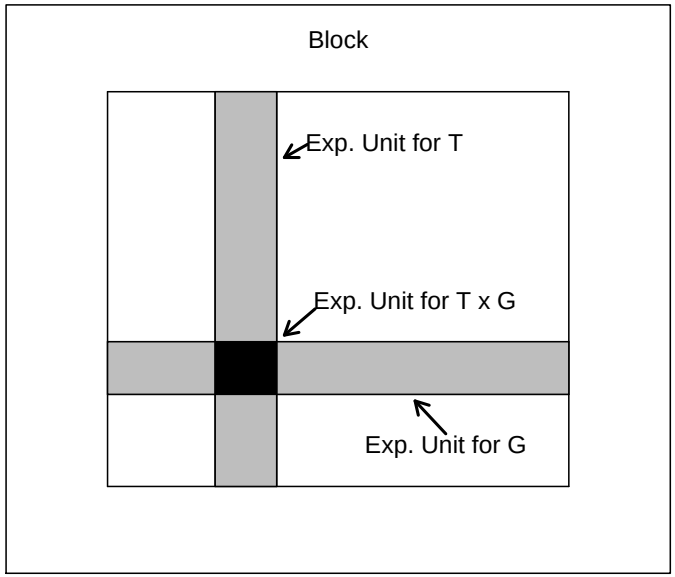
Source	df
Blocks	2
K	2
K x B	4
P	1
P x B	2
K x P	2
K x P x B	4
Total	17

- Potassium(K) randomized$\longrightarrow$ Phosphorus(P) randomized$\downarrow$

- Not a Split Plot - Treatments are Equal

Strip Plot Designs

- The strip plot design actually has three experimental units
- Each treatment and interaction applied to distinct EU



- Correlation is different for the treatments and interaction,
- Higher correlation in the interaction

Strip Plot Bioassay

- Strip plot designs were originally developed to accommodate treatments applied with farm-scale equipment
- They are still relevant today!
  - Cells grown in culture are often sensitive to subtle features in the environment, and may grow better on one side of the plate than another.
  - If a design such as a CRD is used, we must use randomization to control this variation and avoid grouped dilution or serial dilution
  - This can be better handled in a strip plot design

Strip Plot Bioassay

- Samples (dilutions) treated together with multichannel pipettes
- Solution placed *simultaneously* across row or down column

block : C

		1	2	3	4	5	6	7	8	9	10	11	12
ref2	A	1.21	1.27	2.14	2.20	1.44	2.07	1.89	1.32	1.31	2.09	1.50	1.59
hlf2	B	1.34	1.33	2.35	2.32	1.41	2.24	2.03	1.28	1.40	2.43	1.73	1.75
one2	C	1.22	1.35	2.21	2.16	1.36	**2.38**	1.88	1.30	1.36	2.08	1.56	1.58
dub2	D	1.27	1.38	2.25	2.41	1.38	2.02	1.72	1.21	1.32	2.06	1.38	1.52
hlf1	E	1.37	1.45	2.36	2.26	1.44	2.06	2.18	1.30	1.42	2.26	1.81	1.62
dub1	F	1.24	1.35	2.18	2.36	1.35	1.90	1.73	1.23	1.31	2.11	1.46	1.45
ref1	G	1.27	1.38	2.42	2.22	1.43	2.04	1.85	1.34	1.37	2.11	1.49	1.48
one1	H	1.28	1.20	2.02	1.93	1.35	1.93	1.88	1.26	1.32	1.93	1.44	1.51

- Field Layout- 96-well plate
- Four Samples
  - reference, $1/2$, 1, 2
- Twelve Dilutions

Strip Plot Bioassay Anova

	block : C											
ref2 A	1.21	1.27	2.14	2.20	1.44	2.07	1.89	1.32	1.31	2.09	1.50	1.59
hlf2 B	1.34	1.33	2.35	2.32	1.41	2.24	2.03	1.28	1.40	2.43	1.73	1.75
one2 C	1.22	1.35	2.21	2.16	1.36	**2.38**	1.88	1.30	1.36	2.08	1.56	1.58
dub2 D	1.27	1.38	2.25	2.41	1.38	2.02	1.72	1.21	1.32	2.06	1.38	1.52
hlf1 E	1.37	1.45	2.36	2.26	1.44	2.06	2.18	1.30	1.42	2.26	1.81	1.62
dub1 F	1.24	1.35	2.18	2.36	1.35	1.90	1.73	1.23	1.31	2.11	1.46	1.45
ref1 G	1.27	1.38	2.42	2.22	1.43	2.04	1.85	1.34	1.37	2.11	1.49	1.48
one1 H	1.28	1.20	2.02	1.93	1.35	1.93	1.88	1.26	1.32	1.93	1.44	1.51
	1	2	3	4	5	6	7	8	9	10	11	12

- Rows are nested in samples but crossed with blocks

Source	df
Blocks	2
Dilutions (Columns)	11
D × B	22
Rows	7
Samples	3
Rows(in Samples)	4
Rows × Blocks	14
S × B	6
Rows(in Samples) × B	8
D × Rows	77
D × S	33
D × Rows(in Samples)	44
D × Rows × B	154
D × S× B	66
D × Rows(in Samples)× B	88
Total	287

Crossover Designs

- The crossover design combines a bit of everything
  - RCB, Strip Plot, Latin Square
  - Gives tighter control on differences
  - Cost is an assumption on order of treatments

- Simplest Case is Two Period (P_1, P_2) Crossover (SCOD)
  - Two Groups (G_1, G_2) and Two Treatments (T_1, T_2)
  - Data Layout:

	G_1	G_2
P_1	T_1	T_2
P_2	T_2	T_1

or

	T_1	T_2
P_1	G_1	G_2
P_2	G_2	G_1

Simple Crossover

- The groups are "crossed over" to the other treatment
  - Each group receives both treatments, in opposite orders.
  - Each group is its own control
  - We save observations, but get good comparisons
  - What did we give up??

- There is a Washout Period between treatments
  - Assumption of "No Carryover"
  - Assume that groups start P_2 equivalent to starting P_1
  - This is an assumption about interactions

The SCOD is a Split Plot

- Plots=Subjects
- WP Trt = Order, SP Trt = Treatment

Source	df
Order	1
Subjects (in Order)	s-2
Period	1
P × O (Treatments)	1
P × Subjects (in Order)	s-2

- Treatment test confounded with P × O interaction
- To test treatments, need to assume no P × O interaction

The SCOD is a Split Plot - 2

- The test on order (WP level) is testing the carryover effect.
- Both groups receive both treatments
  - Only difference is the order of treatments
  - Nonsignificance means equal carryover, not no carryover!

Source	df
Order	1
Subjects (in Order)	s-2
Period	1
P × O (Treatments)	1
P × Subjects (in Order)	s-2

- Without Order Effect
  T_1 vs. T_2
- With Order Effect
  T_1 after T_2
  T_2 after T_1

- All of the tests in the SCOD are t-tests

Exercise Crossover Design

- Effects of aerobic exercise on riboflavin requirements
- 12 subjects, NE/E or E/NE, where NE=no exercise, E=exercise

Order	Subject	UrRibo	Order	Subject	UrRibo
NE	1	29.5	E	7	14.0
Period 1	⋮	⋮		⋮	⋮
	6	20.4		12	15.0
E	1	31.6	NE	7	26.3
Period 2	⋮	⋮		⋮	⋮
	6	11.3		12	27.8

Source	df	p-value
Order	1	0.250
Subjects (in Order)	10	
Period	1	0.813
Period $\times$ Order (Trt)	1	0.004
Split Plot Error	10	

- Significant Treatment Effect
- If no Period $\times$ Order interaction

Three Period Crossover

- Crossover design starts to get unwieldy here
- Beyond three treatments is probably not a good idea
  - Multiple washout periods
  - Assumption of no carryover effect becomes tenuous
- A Possible Layout

	Subjects					
	1	2	3	4	5	6
1	a	b	c	c	a	b
Period 2	b	c	a	b	c	a
3	c	a	b	a	b	c

- Note the two orthogonal Latin squares

Three Period Crossover Anova

	Subjects					
	1	2	3	4	5	6
Period 1	a	b	c	c	a	b
Period 2	b	c	a	b	c	a
Period 3	c	a	b	a	b	c

Source	df	SS
Order	5	6252.4
Period	2	1053.8
Period × Order	10	13056.2
Drug	2	2276.8
Residual	8	10779.4

- Treatment is now only a piece of the P × O interaction
- Subjects and Order are completely confounded here
  - The design is, in fact, an RCB and not a split plot

Repeated Measures

- Typically multiple measurements on a subject over time
- If Treatment is applied to the Subjects
  - Subjects = Whole Plots, Time = SP Trt
- Blood Pressure response to High/Low Ca Diets

Treatment	Subject	Time		
		1	2	3
HighCa	1	133	141	100
	⋮	⋮	⋮	⋮
	5	171	142	128
LowCa	6	104	139	153
	⋮	⋮	⋮	⋮
	10	147	167	157

Source	df	MS	p-value
Treatment	1	1153.2	0.2418
Whole Plot Error	8	721.6	
Time	2	171.6	
Trt × Time	2	2514.1	
Split Plot Error	16	110.7	

Repeated Measures Anova

- Equicorrelation less tenable
- Plausible correlation

$$\mathrm{Corr}(Y_{ijk}, Y_{ijk'}) = \rho^{|k-k'|}$$

- Invalid SP F-tests

Source	df	MS	p-value
Treatment	1	1153.2	0.2418
Whole Plot Error	8	721.6	
Time	2	171.6	
Trt × Time	2	2514.1	
Split Plot Error	16	110.7	

Some Options

- We can use an approximate F-test.
  - Such tests are usually conservative
- Hotelling's T^2 is valid test against any covariance structure.
  - Typically a substantial loss of power
- The repeated measures can be summarized

Summarizing the Repeated Measure

- Suppose the interest is in the change in BP over time
- Fit a linear regression to each subject
  - Use the slope as the response
- Does not assume linear response, just summarizes the trend

Slopes for each subject

HighCa				LowCa			
1	2	$\cdots$	5	6	7	$\cdots$	10
-16.5	-14.0	$\cdots$	-21.5	24.5	25.5	$\cdots$	5.0

Source	df	MS	F	p-value
Treatments	1	2512.23	41.619	0.0002
Within	8	60.36		

- Valid anova - the subjects are independent, good power
- The anova on the slopes is very significant

An Example

Medical Split Plot

- Comparing the performance of a new type of catheter to the standard type
- The response to be measured is the pressure inside the catheter
- Sensors placed at two points along the catheter, Distal and Proximate.
- For each patient the measurements were to be taken at two organ ducts

An Example

Medical Split Plot - 2

For each patient

Sensor Location

Distal		Proximate	
	Organ Duct		Organ Duct
Catheter Type		Catheter Type	

- The treatment design is a $2 \times 2 \times 2$ factorial
  - 30 patients available
  - Each patient will have these 8 pressure measurements taken

Randomization

Medical Split Plot - 3

- There are a number of ways to carry out the randomization
- Here are three:

(1) Randomize throughout the $2 \times 2 \times 2$ factorial.

(2) Choose an organ duct, then randomize throughout Catheter $\times$ Sensor combinations.

(3) Choose a catheter type, randomize the organs within catheter, then randomize sensor in organ.

Designs

Medical Split Plot - 4

Let O denote Organ, C the Catheter, S the Sensor and P the patient.

- (1): Randomize throughout treatments, P = blocks.
  - RCB design.
- (2): Randomize throughout $C \times S$ in O, P = blocks.
  - Split plot design, whole plots in RCB
  - O = WP trt $\circ$ $C \times S$ = split plot trt
- (3): Randomize C, then O in C, then S in O , P = blocks.
  - Split split plot design, whole plots in RCB
  - C = WP trt $\circ$ O = SP trt $\circ$ S = SSP trt.

Medical Split Plot - RCB

- Design (1) is an RCB
- Treatment effects are tested against their interaction with blocks
- With 30 blocks there is no need for pooling interaction terms.

Source	df
P	29
O	1
C	1
S	1
$O \times C$	1
$O \times S$	1
$C \times S$	1
$O \times C \times S$	1
$P \times O$	29
$P \times C$	29
$P \times S$	29
$P \times O \times C$	29
$P \times O \times S$	29
$P \times C \times S$	29
$P \times O \times C \times S$	29

Medical Split Plot - SP

- Design (2) is split plot, whole plot treatment in blocks P

- Above the line O is tested against P $\times$ O.

- Below the line each effect is tested against its interaction with P

- Again, with 30 blocks there is no need to pool interactions.

Source	df
P	29
O	1
$P \times O$	29
C	1
S	1
$C \times S$	1
$C \times O$	1
$S \times O$	1
$C \times S \times O$	1
$P \times C$	29
$P \times S$	29
$P \times C \times S$	29
$P \times C \times O$	29
$P \times S \times O$	29
$P \times C \times S \times O$	29

Medical Split Plot - SSP

- Design (3) is a split split plot whole plot in blocks P.

- As before, everything is tested against its interaction with P.

Source	df
P	29
C	1
$P \times C$	29
O	1
$O \times C$	1
$O \times P$	29
$O \times C \times P$	29
S	1
$S \times C$	1
$S \times O$	1
$S \times O \times C$	1
$S \times P$	29
$S \times C \times P$	29
$S \times O \times P$	29
$S \times O \times C \times P$	29

Medical Split Plot - Conclusions

a. Design (1) - RCB

- ▷ If interest in all treatments equal

b. Design (2) - SP

- ▷ Better information on C and S

c. Design (3) - SSP

- ▷ Good information on C, better information on S

- ▶ Need to also consider physical limitations
  - ▷ Some randomizations may not be feasible

Chapter 6: Confounding in Blocks

It is easy to conduct an experiment in such a way that no useful inferences can be made...

William Cochran and Gertrude Cox

Experimental Designs

Introduction

- Thus far, we have only looked at *complete* designs
  - Every treatment has appeared in every block.
- This is the best situation, and gives the best information for treatment comparisons.
  - In many situations we cannot put every treatment in every block
  - Often due to time, money, or physical constraints of the experiment
  - For example, a microarray two-dye chip is restricted to two treatments per block.
- In these cases the design becomes *incomplete*, and there is confounding

Problems from Incomplete Designs

- Treatment comparisons are confounded with block effects
  - Block differences may affect treatment comparisons
  - Block variances could inflate treatment variance
- Example: Effects of diet on BP in African-American males

  A = amount of fruits and vegetables in the diet (low/high)
  B = amount of fat in the diet (low/high)
  C = amount of dairy products in the diet (low/high)

- Eight Treatment Combinations, 2^3 factorial
  - Only four treatment combinations can be run at one time
  - The experiment will be run in two blocks

Confounding in Blocks

Block

1	
a	b
c	abc

2	
(1)	ab
ac	bc

- ABC confounded with blocks
- Block 1 = high, Block 2 = low
- Other effects balanced between blocks

- Notation: Present/Absent

		Effect						
Block	Trt. Comb	A	B	C	AB	AC	BC	ABC
1	a	+	-	-	-	-	+	+
1	b	-	+	-	-	+	-	+
1	c	-	-	+	+	-	-	+
1	abc	+	+	+	+	+	+	+
2	(1)	-	-	-	+	+	+	-
2	ab	+	+	-	+	-	-	-
2	ac	+	-	+	-	+	-	-
2	bc	-	+	+	-	-	+	-

Confounding in Blocks -2

- Any Effect can be confounded with blocks

Block

1	
(1)	a
bc	abc

2	
b	c
ab	ac

- BC confounded with Blocks

- If we run both blocks
  - Partial information on BC and ABC
- If we confound all effects
  - Need 14 blocks
  - BIBD

Balancing the whole thing

Block Pair		Confounded Effect
a ab ac abc		A
(1) b c bc		
b ab bc abc	*	B
(1) a c ac	*	
c ac bc abc	*	C
(1) a b ab	*	

Block Pair		Confounded Effect
a b ac bc		AB
(1) c ab abc		
a c ab bc		AC
(1) b ac abc		
b c ab ac	*	BC
(1) a bc abc	*	
a b c abc		ABC
(1) ab ac bc		

- If Block Pairs Joined $\Rightarrow$ RCB
- Partial Information on Block Interactions
  - A $\times$ Block only from $*$

Anova for the whole thing

A × Block Interaction

b ab bc abc	*	B
(1) a c ac	*	
c ac bc abc	*	C
(1) a b ab	*	
b c ab ac	*	BC
(1) a bc abc	*	

Source	df
Blocks	13
Trts	7
Trts × Blocks	7 × 5 = 35
Total	55

- Each Interaction Effect Estimated from Six Blocks
- RCB: 7 df shifted from Blocks to T × B
- This is a BIBD

Balanced Incomplete Blocks

- Properties of a BIBD
  - Every treatment is estimated with the same variance
  - Every $contrast$ is estimated with the same variance.
  - Contrast variance is free of the block variance
- A BIBD with t treatments and b blocks satisfies:
  - Each block has k treatments $(k < t)$,
  - Each treatment appears in r blocks $(r < b)$
  - Every pair of treatments appears together λ times

BIBD Illustrations

- The BIBD is characterized by the five numbers (t, k, b, r, λ)

Block			
1	2	3	4
A	A	A	B
B	B	C	C
C	D	D	D

- $t = 4, k = 3, b = 4, r = 3, \lambda = 2$

- BIBD Defining Equations
  - $rt = bk$
  - $\lambda(t-1) = r(k-1)$
- Derived by counting EUs

Model and Distribution Assumptions

- BIBD model is essentially equivalent to the RCB model

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij},$$

  - $\varepsilon_{ij} \sim$ iid $\mathsf{N}(0, \sigma^2_\varepsilon)$, $\beta_j \sim$ iid $\mathsf{N}(0, \sigma^2_\beta)$ independent
  - The difference is in the index set

Source	df	SS	MS	F
Blocks	b-1	SS(Blocks)	MS(Blocks)	
Treatments	t-1	SS(Trts)	MS(Trts)	$\frac{\text{MS(Trts)}}{\text{MS(T} \times \text{B)}}$
T × B	bk-b-t+1	SS(T × B)	MS(T × B)	
Total	bk-1	SS(Total)		

- Test on treatments is the same as in the RCB
- $\text{MS(T} \times \text{B)}$ is an unbiased estimator of σ^2_ε

Estimating Contrasts

- The least squares estimates of τ_i are

$$\hat{\tau}_i = \frac{k}{\lambda t}\left(r\bar{y}_i - \sum_{j \in J_i} \bar{y}_j\right)$$

- and, as they are least squares, unbiased estimators of τ_i.

- Treatment variances are free of block variances

$$\mathrm{Var}(\hat{\tau}_i) = \frac{k}{\lambda t}\left(\frac{t-1}{t}\right)\sigma_\varepsilon^2,$$

  - The real advantage of the BIBD
  - Note the important role played by λ

A Sad Example

- Project to relate gene expression genes to substantiality of crops (potatoes)
- Two crossed factors
  - Photoperiod (P) and bioactive Tuber Inducing Factor (TIF)
  - Each factor at two levels (2=high and 1=low)
- Using an `Agilent` microarray chip, a two-dye system.
  - Two treatments can be applied to each array
- Experimenter ran his own experiment

All Four Pairs

- Experiment that was done - The Case of the Missing Pairs!

		Effect			
Array	Trt. Comb	P	T	PT	Confounded
1	(1)	-	-	+	T
	p	+	-	-	
2	p	+	-	-	P
	pt	+	+	+	
3	(1)	-	-	+	P
	t	-	+	-	
4	(1)	-	-	+	PT
	pt	+	+	+	

- Damage Control
- Treatment contrasts not free of block effects
  - Design not balanced
  - Hard to separate treatment effects

All Six Pairs

- Experiment that should have been done
- BIBD: $t = 4, k = 2 \Rightarrow r = 3, b = 6, \lambda = 1$

		Effect			
Array	Trt. Comb	P	T	PT	Confounded
1	(1)	-	-	+	T
	p	+	-	-	
2	p	+	-	-	P
	pt	+	+	+	
3	(1)	-	-	+	P
	t	-	+	-	
4	(1)	-	-	+	PT
	pt	+	+	+	
5	p	+	-	-	PT
	t	-	+	-	
6	t	-	+	-	T
	pt	+	+	+	

Fractions of Factorials

- BIBD cycles and confounds each effect with blocks
  - In the end, we can recover information about each effect
- If we run only a piece of the design, however, some effects will not be estimable
  - There will be a loss of information
  - Some effects will be confounded
  - This is the idea behind *Fractional Factorial Designs*.
- The key is to understand the confounding, so that the important information is not lost

A simple fractional factorial

a	ab	ac	abc

- $1/2$ replication of a 2^3 factorial
- Not a particularly good design
- No good information on main effects

	Effect						
Trt. Comb	A	B	C	AB	AC	BC	ABC
a	+	-	-	-	-	+	+
ab	+	+	-	+	-	-	-
ac	+	-	+	-	+	-	-
abc	+	+	+	+	+	+	+

- Better for B: (1) b ac abc
- Here B $\sim$ ABC

A $\sim$ Blocks
B $\sim$ AB
C $\sim$ AC
BC $\sim$ ABC

- Subject matter $\Rightarrow$ what can be confounded
- Careful planning needed for appropriate inference
- No within error here

Alias Sets and Modular Arithmetic

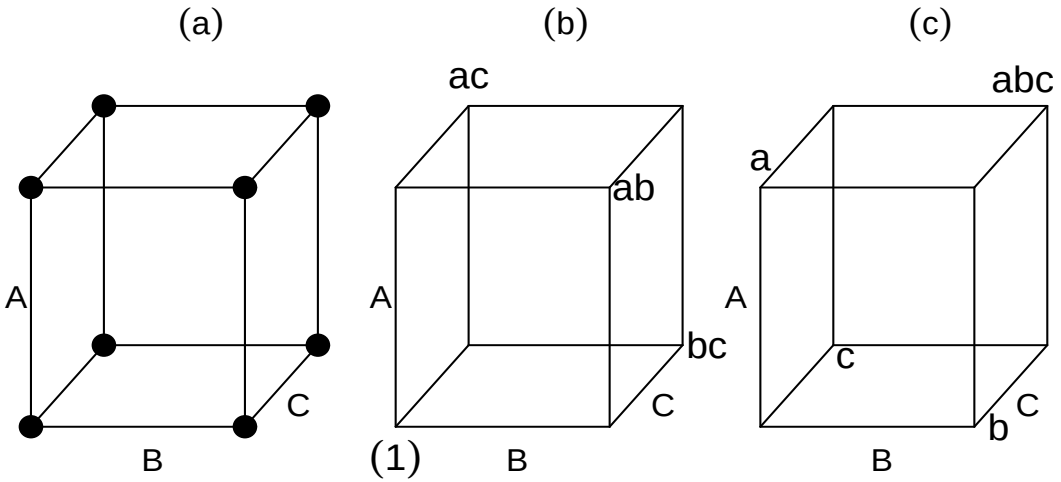
- Alias set = { Trt Comb. estimated by the same contrast }
- Alias sets and blocks are found using modular arithmetic
  - To confound B and ABC, write $x_2 = x_1+x_2+x_3$ or $x_1+x_3 = 0$

$x_1 + x_3 = 0$		$x_1 + x_3 = 1$	
000	(1)	100	a
010	b	001	c
101	ac	110	ab
111	abc	011	bc

Source	df
Blocks	1
A	1
B	1
C	1
Residual	3

- Complete factorial in blocks
- Each block a 1/2 rep
- Each block has the same information

Running the Factorial



- Main effects confounded with interactions

- Fractional factorial typically run as a CRD or an RCB
- Add assumptions that certain interactions are zero
  - Necessary in order to get estimates of the main effects
- Effect estimates more precise than mean estimates

Variations on a Theme

- Some examples that go a little beyond the designs that we have been discussing
  - Balanced Lattice Designs
  - Latin Squares/Frac. Factorials/Split Plots
  - Loops and Reference Designs
- Back to BIBDs

Balanced Lattice Designs

- An incomplete block design with each treatment appearing r times is resolvable if the blocks can be divided into r groups with each group having a complete replication of the treatments.
  - RCB yes, BIBD no.
- Balanced Lattice Square
  - Number of treatments, t is a square
  - A set of $\sqrt{t}+1$ orthogonal Latin squares of side t exists
  - Each pair of treatments appears once in each row and once in each column

Balanced Lattice Design, $t = 9$

Rep 1

Col. \ Row	1	2	3
1	6	4	5
2	3	1	2
3	9	7	8

Rep 2

Col. \ Row	1	2	3
1	4	2	9
2	3	7	5
3	8	6	1

Rep 3

Col. \ Row	1	2	3
1	8	4	3
2	6	2	7
3	1	9	5

Rep 4

Col. \ Row	1	2	3
1	7	1	4
2	9	3	6
3	8	2	5

Source	df
Reps	$r-1$
Columns	$r(k-1)$
Rows	$r(k-1)$
Treatments	$t-1$
Residual	$r(k-1)(k-1)-(t-1)$
Total	$rt-1=rk^2-1$

- Rows are BIBD
- Columns are BIBD

  $\hat{\tau}_i = \frac{t-1}{(c-1)^2}(\bar{y}_i - \bar{y}_{iR} - \bar{y}_{iC} + \bar{\bar{y}})$
- Variance Free of Block Effects

- Experiment run in "manageable" blocks

Latin Squares and Fractional Factorials

- In some situations a Latin square is a fractional factorial
- Kerr *et al.* (2000) describe a microarray Latin square
  - mRNA from liver tissue was compared to muscle tissue

	Array	
Dye	1	2
Red	Liver	Muscle
Green	Muscle	Liver

Confounding structure:

mean	$\sim$	ADT	G	$\sim$	ADTG
A	$\sim$	DT	AG	$\sim$	DTG
D	$\sim$	AT	DG	$\sim$	ATG
T	$\sim$	AD	TG	$\sim$	ADG

- Right = Left +G (G crossed)
- Valid inference $\Rightarrow$ Right side effects are 0

$$\log Y_{ijkg} = \mu + A_i + D_j + T_k + G_g + (AG)_{ig} + (TG)_{kg} + \varepsilon_{ijkg}$$

Microarray Latin Square

- The Latin square is a $1/2$ rep with $x_1 + x_2 + x_3 = 0$ or 1

			Effect			
Trt.	Comb		Array	Dye	Tissue	D × T
1	R	L^*	-	-	-	+
1	R	M	-	-	+	-
1	G	L	-	+	-	-
1	G	M^*	-	+	+	+
2	R	L	+	-	-	+
2	R	M^*	+	-	+	-
2	G	L^*	+	+	-	-
2	G	M	+	+	+	+

Source	df	SS
Array	1	13.675
Dye	1	0.127
Treatment	1	5.577
Gene	99	87.908
A × G	99	21.550
T × G	99	46.873
Residual (D × G)	99	3.471

- $*$ Treatment Combinations were run
- Threeway Interaction confounded with Blocks

Reference and Loop Designs

- With two-dye systems the experiment is an incomplete block design (unless there are only two treatments)
- With t treatments, a BIBD we would need $\binom{t}{2}$ microarrays.
  - There is sometimes concern about dye bias
  - To control this, the experiment would include a dye-swap

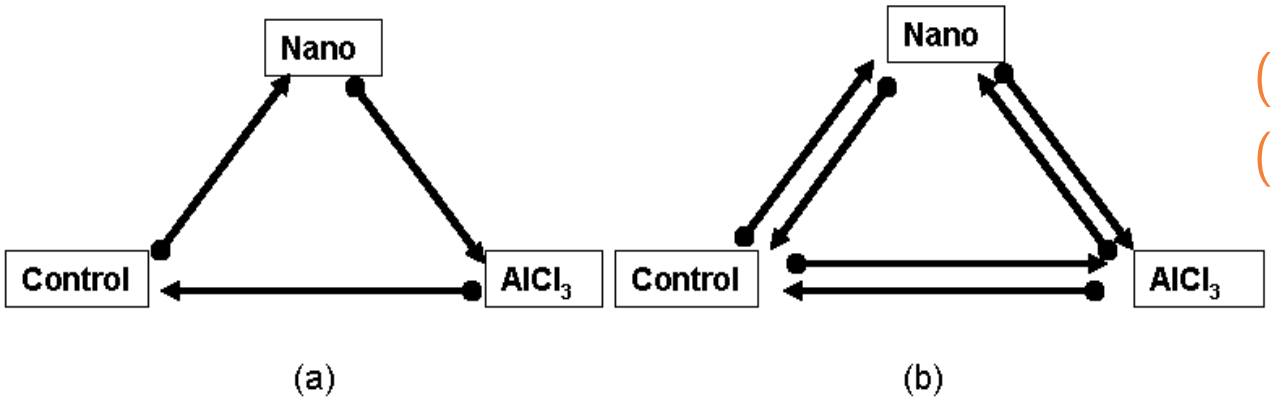
Reference Design		
Block 1	Block 2	Block 3
A	B	C
R	R	R

BIBD		
Block 1	Block 2	Block 3
A	B	C
B	C	A

- Unfortunately, the Reference Design became popular
- "fully half of the data are dedicated to an extraneous sample"

Experiment: Effect of Aluminum on Zebrafish

- Three treatments : Control, $AlCl_3$(aluminum chloride) and Nano (aluminum nano particles)
  - Treatments applied to tanks holding the Zebrafish
  - RNA extracted; microarray analysis with two dye system
- Possible Designs (• = red dye ▲ =green dye)



(a) = Loop

(b) = Double Loop

Reference and Loop Variance

- Loops: Balance the Dye Effect
- One gene model: $y_{ijk} = \mu + \tau_i + \beta_j + D_k + \varepsilon_{ijk}$

Dye	Reference			Loop		
Green	Ref.	Ref.	Ref.	Cont.	Nano	$AlCl_3$
Red	Cont.	$AlCl_3$	Nano	Nano	$AlCl_3$	Cont.

$$\text{Reference Design} : \text{Var}(\hat{\tau}_i - \hat{\tau}_{i'}) = 2\sigma_\varepsilon^2 + 2\sigma_\beta^2$$

$$\text{Loop Design} : \text{Var}(\hat{\tau}_i - \hat{\tau}_{i'}) = \sigma_\varepsilon^2 + \frac{1}{2}\sigma_\beta^2$$

- Yes, they did the reference design

Reference and Loop Anova

- Modelling many genes simultaneously

$$y_{ijkg} = \mu + \tau_i + \beta_j + D_k + G_g + (\tau G)_{ig} + (\beta G)_{jg} + \varepsilon_{ijkg}$$

Reference

Source	df
Blocks	2
Trts	3
Genes	n-1
T × G	3(n-1)
B × G	2(n-1)
Residual	0
Total	6n-1

Loop

Source	df
Blocks	2
Trts	2
Dye	1
Genes	n-1
T × G	2(n-1)
B × G	2(n-1)
Residual	n-1
Total	6n-1

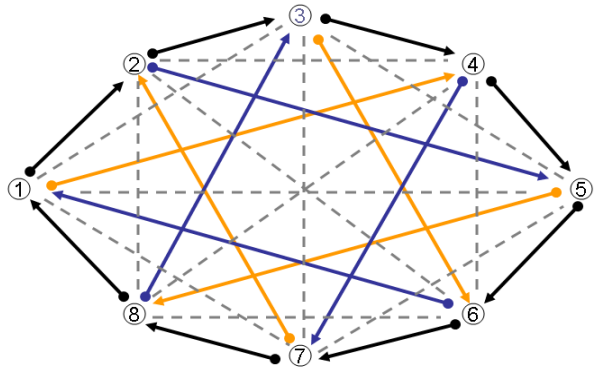
- Gene test OK
- Reference: wasted df in T × G
- T and T × G tested with Residual

Loops and Beyond

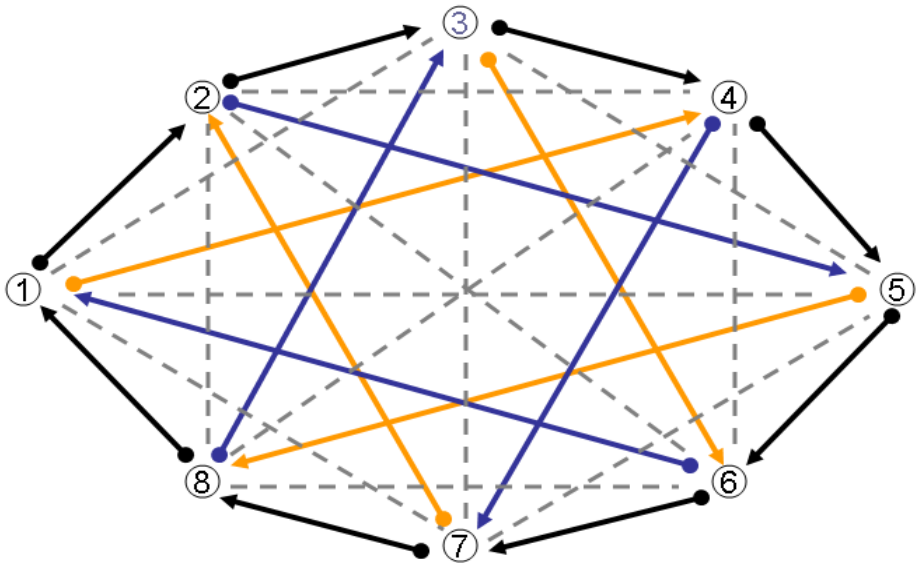
- Loop Designs
  - Balance dyes
  - Provide good comparisons between adjacent treatments
- Can add blocks to improve non-adjacent comparisons

RNA from Eight Avocado Tissues

Number	Name	Number	Name
1	medium bud	5	petal
2	small bud	6	stamen
3	leaf	7	carpel
4	sepal	8	fruit



Loops, Augmented Loops, and BIBDs



- Black = Loop
- Black + Orange+Blue = Augmented Loop
- All = BIBD
- Variances ↓ as we add lines

- Adjacent comparisons better than non-adjacent
- Trts in the same block have more precise comparisons
  - Except for BIBD, Block variance part of Trt variance

Example

BIBD

- Three factors, A, B, and C, each at two levels in a BIBD
- The data are

Rep 1				Rep 2				Rep 3				Rep 4			
B1		B2		B3		B4		B5		B6		B7		B8	
(1)	10	a	17	(1)	11	a	8	(1)	6	b	9	a	17	(1)	9
ab	17	b	12	b	9	ab	9	a	15	ab	14	b	13	ab	15
c	9	ac	19	ac	16	c	6	bc	8	c	7	c	9	ac	17
abc	10	bc	11	abc	16	bc	2	abc	1	ac	14	abc	16	bc	14

- For each rep, we can see which effect is confounded with blocks.

	Rep			
	1	2	3	4
Confounded	AB	AC	BC	ABC

- The easiest way to do this question is to stare at an effect table..

Confounding

BIBD - 2

Rep 1				Rep 2				Rep 3				Rep 4			
B1		B2		B3		B4		B5		B6		B7		B8	
(1)	10	a	17	(1)	11	a	8	(1)	6	b	9	a	17	(1)	9
ab	17	b	12	b	9	ab	9	a	15	ab	14	b	13	ab	15
c	9	ac	19	ac	16	c	6	bc	8	c	7	c	9	ac	17
abc	10	bc	11	abc	16	bc	2	abc	1	ac	14	abc	16	bc	14

- In Rep 3 the BC interaction is confounded with blocks
- This can be seen from the following contrast table:

	A	B	C	BC
(1)	−	−	−	+
a	+	−	−	+
bc	−	+	+	+
abc	+	+	+	+

	A	B	C	BC
b	−	+	−	−
ab	+	+	−	−
c	−	−	+	−
ac	+	−	+	−

Analysis

BIBD - 3

- Calculate the anova table and test the treatments.

```
summary(aov(Y ~Rep+Block+A*B*C,data=aovdata))
```

```
            Df  Sum Sq Mean Sq F value    Pr(>F)
Rep          3 123.750  41.250  5.9302  0.005845 **
Block        4 125.750  31.438  4.5196  0.011398 *
A            1 200.000 200.000 28.7526 5.174e-05 ***
B            1   8.000   8.000  1.1501  0.298516
C            1   4.500   4.500  0.6469  0.432316
A:B          1  22.042  22.042  3.1688  0.092938 .
A:C          1  15.042  15.042  2.1624  0.159687
B:C          1   1.500   1.500  0.2156  0.648270
A:B:C        1  16.667  16.667  2.3961  0.140056
Residuals   17 118.250   6.956
```

- Only partial information on the interactions
- Residual is a mix of all of the Trt $\times$ Block interactions.
- 17 df for Residual?

Analysis

BIBD - 4

- Lost degrees of freedom from confounding with blocks

Rep 1				Rep 2				Rep 3				Rep 4			
B1		B2		B3		B4		B5		B6		B7		B8	
(1)	10	a	17	(1)	11	a	8	(1)	6	b	9	a	17	(1)	9
ab	17	b	12	b	9	ab	9	a	15	ab	14	b	13	ab	15
c	9	ac	19	ac	16	c	6	bc	8	c	7	c	9	ac	17
abc	10	bc	11	abc	16	bc	2	abc	1	ac	14	abc	16	bc	14

Without Blocks

Source	df
Reps	3
Trts	7
Trts × Reps	21
Total	28

With Blocks

Source	df
Reps	3
Trts	7
Trts × Reps	21
Trts × Blocks in Reps	4
Residual	17
Total	28

Designs Illustrated

- A small catalog of designs for review
- Four treatment combinations: (1), a, b, and ab
- Look at Layout and Randomization
  - 16 observations
  - Each design has 15 total degrees of freedom
- How many ways can you count to 15?

Completely Randomized Design

Layout

a	(1)	b	a
b	a	ab	b
a	ab	(1)	ab
(1)	b	ab	(1)

Anova

Source	df
Treatments	3
A	1
B	1
A × B	1
Within Error	12
Total	15

- The within error is model independent
- Difficult design to run
  - Experimental conditions must be reconstructed every time
- Disadvantage: One "Block" - Limits scope of inference

RCB - no subsampling

Layout

Block					
	1	a	(1)	b	ab
	2	b	a	ab	(1)
	3	a	ab	(1)	b
	4	(1)	b	ab	a

Anova

Source	df
Blocks	3
Treatments	3
T × B	9
Total	15

- Typically easier to run than a CRD
- Here we pooled all of the T × B interactions
- There is no test on the interaction in this model

RCB - with subsampling

Layout

Block								
	1	a	(1)	b	ab	a	ab	(1)	b
	2	b	a	ab	(1)	(1)	b	ab	a

Anova

Source	df
Blocks	1
Treatments	3
T × B	3
Within Error	8
Total	15

- The test on treatments is not as good as previous RCB
- If the observations within a block
  - Are true (not technical) replications
  - The within error can be used to test T × B

Latin Square

Layout

		Columns			
		1	2	3	4
Rows	1	(1)	b	a	ab
	2	ab	a	b	(1)
	3	b	(1)	ab	a
	4	a	ab	(1)	b

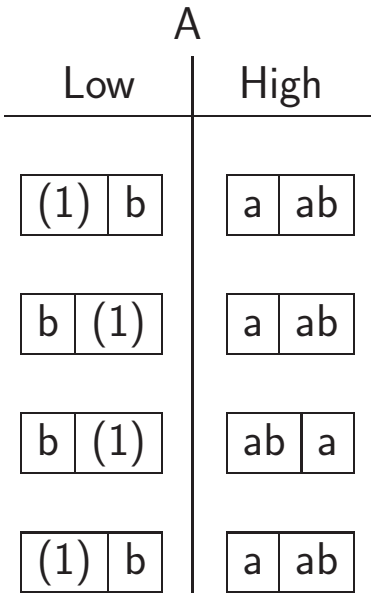
Anova

Source	df
Rows	3
Columns	3
Treatments	3
Residual	6
Total	15

- The design controls two gradients
- Assumption of no interactions
  - Needed for a good test on treatments
  - Test can be conservative

Split Plot - CRD on Whole Plots

Layout



Anova

Source	df
A	1
Reps (in A)	6
B	1
A × B	1
(B × Reps) in A	6
Total	15

- SP Trt. B is randomized on the whole plots
- WP error, Reps (in A), tests A
- SP error, (B × Reps) in A, tests everything below the line.

Split Plot - RCB on Whole Plots

Layout

	A: Low	A: High
Block 1	(1) b	a ab
Block 2	b (1)	a ab
Block 3	(1) b	ab a
Block 4	(1) b	ab a

Anova

Source	df
Blocks	3
A	1
A × Blocks	3
B	1
A × B	1
Split Plot Error	6
Total	15

- B randomized within the levels of A
- All factors are crossed, in contrast to CRD SP design
- One SP error $\Rightarrow$ Assume no Block $\times$ SP interaction

Strip Plot

Layout

Block 1

b	ab
(1)	a

Block 2

(1)	a
b	ab

Block 3

ab	b
a	(1)

Block 4

ab	b
a	(1)

Anova

Source	df
Blocks	3
A	1
A × Blocks	3
B	1
B × Blocks	3
A × B	1
A × B × Blocks	3
Total	15

- In each block
  - A is randomized in columns
  - B is randomized in rows
- Separately, this is an RCB on each of A and B.

Confounding in Blocks - No Interaction Test

Layout

	I	a | b	(1) | ab
Reps	II	(1) | ab	b | a
	III	a | b	ab | (1)
	IV	b | a	ab | (1)

Anova

Source	df
Reps	3
Blocks (in Reps)	4
A	1
B	1
Residual	6
Total	15

- Not a great design
  - Unless there is no chance of A $\times$ B being significant
- In each rep the interaction is confounded with blocks
  - So there is no test on interaction

Confounding in Blocks - With Interaction Test

Confounded with Blocks	Rep	Layout	
AB	I	a | b	(1) | ab
AB	II	(1) | ab	a | b
B	III	(1) | a	b | ab
A	IV	(1) | b	a | ab

Anova

Source	df
Reps	3
Blocks (in Reps)	4
A	1
B	1
A × B	1
Residual	5
Total	15

- Interaction: Information from two Reps
  Main Effects: Information from three Reps
  - Reps II, III and IV (or I, III and IV) are a BIBD
  - $t = 4$, $b = 6$, $\lambda = 1$, and $k = 2$

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Thanks for your attention

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http://www.stat.ufl.edu/∼casella/StatDesign