

# Lecture 5

- Brief Review
- Treatment Structure
- ANCOVA
- Split Plot Designs

# Some Good References

- Chapter 2 of Scheiner and Gurevitch(2001) Book is very good on testing and power analysis.
- Chapter 4 of Scheiner and Gurevitch(2001) book is very good on basic experimental designs and the split plot design.
- Chapter 8 Scheiner and Gurevitch(2001) has a lot on repeated measures
- Steel, Torrie and Dickey (1997) Principles and Procedures of Statistics. Third Edition. McGraw Hill. Ch 16 has Split plots and a little on repeated measures. Chapter 17 is very good on the analysis of covariance. In fact the whole book is good!

# Key Design Concepts

## Earlier Work- Randomization Method

### Completely Random Design

Treats randomized over all the exptl units

### Randomized Complete Block Design

Treats randomized within each block.

## Today's Lecture- Treatment Designs- How to choose the Treatment Set

Simple Treatment Structure

Factorial Treatment Structure

# Treatment Structures

- **Simple Treatment Structure**

Simple set of treatments perhaps with one a control. There could be some structure amenable to orthogonal contrasts in some cases.

- **Factorial Treatment Structure**

Factor 1 - k levels (say 3)

Factor 2 – j levels (say 2)

$k \times j = 3 \times 2 = 6$  treatments total

# Treatment Designs: Simple Structure

- Large Scale means field studies often have to have a very few treatments
- Small Scale Microcosms and Mesocosms may have more complex treatment structures
- Very careful thought needed based on what you already know about the problem.
- Often one views one of the treatments as a control.

# Treatment Designs: Simple Structure

- Feeding Study with fish species in tanks or ponds. Control might be a standard diet, the other treatments may be increasing levels of some nutrient.
- Burn Study, Control may be no burn, and the other treatments different burn regimes( ie. every year, every other year, every 3 years). Total treats (4 treats {C, 1yr, 2yr, 3yr}).

# Treatment Designs: Simple Structure

Simple set of treatments often with one a control. There could be structure amenable to contrasts.

Haddad Expt treatment structure is a good example of this. 3 treatments total as follows:

1. Connected Patch (C)

Isolated Patches of 2 kinds

2. Isolated Winged patch (IW)

3. Isolated Rectangular Patch (IR)

# Simple Treatment Structure: Orthogonal Contrasts

- If there are 3 treatments there are only 2 df for the treatments
- This means only 2 orthogonal or independent contrasts are possible.
- One useful set of orthogonal contrasts is the following set.

<b>C</b>	<b>IW</b>	<b>IR</b>
<b>2</b>	<b>-1</b>	<b>-1</b>
<b>0</b>	<b>1</b>	<b>-1</b>

## First Contrast

Connected Patches vs Isolated Patches

## Second Contrast

Winged vs Rectangular Isolated Patches

**Note:** Ecologists may be resistant to using orthogonal contrasts here and want to just compare all 3 treatments in pairs. While these contrasts are not orthogonal they are often done.

# Treatment Designs: Simple Structure

Trade off number of treats vs. number of reps.

- 4 Treats and 6 reps = 24 exp units
- 3 treats and 8 reps = 24 exp units
- 2 treats and 12 reps = 24 exp units

# Factorial Treatment Structure

- Very important treatment design structure for where there are 2 **or more** factors at multiple levels. Factor 1 - k levels (say 3) Factor 2 – j levels (say 2)
- $k \times j = 3 \times 2 = 6$  treatments total
- **Example:** Hypothetical New Corridor Experiment where Factor 1: patch sizes (3), and Factor 2: patch connectivity (2, 1 connected and 1 unconnected)
- There would be  $3 \times 2 = 6$  treatments total.

# Factorial Treatment Structure

- Note that **main effects** of factors and their **interaction** are now impt. Are the factors additive in effect or not? (ie if not they are interacting)
- Each component has a line in the expanded ANOVA table and the df of each is also important. If **additive** the interaction test is not significant whereas if **non additive** then the interaction F test will be significant.
- **Crucial to study nature of interaction graphically. We consider in the next slide.**

# Factorial Treatment Structure

## Randomized Block Design ANOVA

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Treats	5			
Blocks	4			
<u>Residual</u>	<u>20</u>			
<u>Total</u>	<u>29</u>			

5 Blocks, 6 treatments in a 3x2 factorial design.

# Factorial Treatment Structure

## Partitioning the Treat df and SS

<u>Source</u>	<u>df</u>	<u>SS</u>
Treats	5	A-Main Effect of Factor A B-Main Effect of Factor B AB-Interaction of A and B
A	2	
B	1	
AB	2	

# Factorial Treatment Structure

## Randomized Block Design ANOVA

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
A	2			
B	1			
AB	2			
Blocks	4			
<u>Residual</u>	<u>20</u>			
<u>Total</u>	<u>29</u>			

5 Blocks, 6 treatments in a 3x2 factorial design.

# Factorial Designs: Interaction Diagram

We will consider the simple case of a 2x2 factorial and use a diagram from the book by Steel et al. (1997)

# Factorial Designs: Interaction Diagram

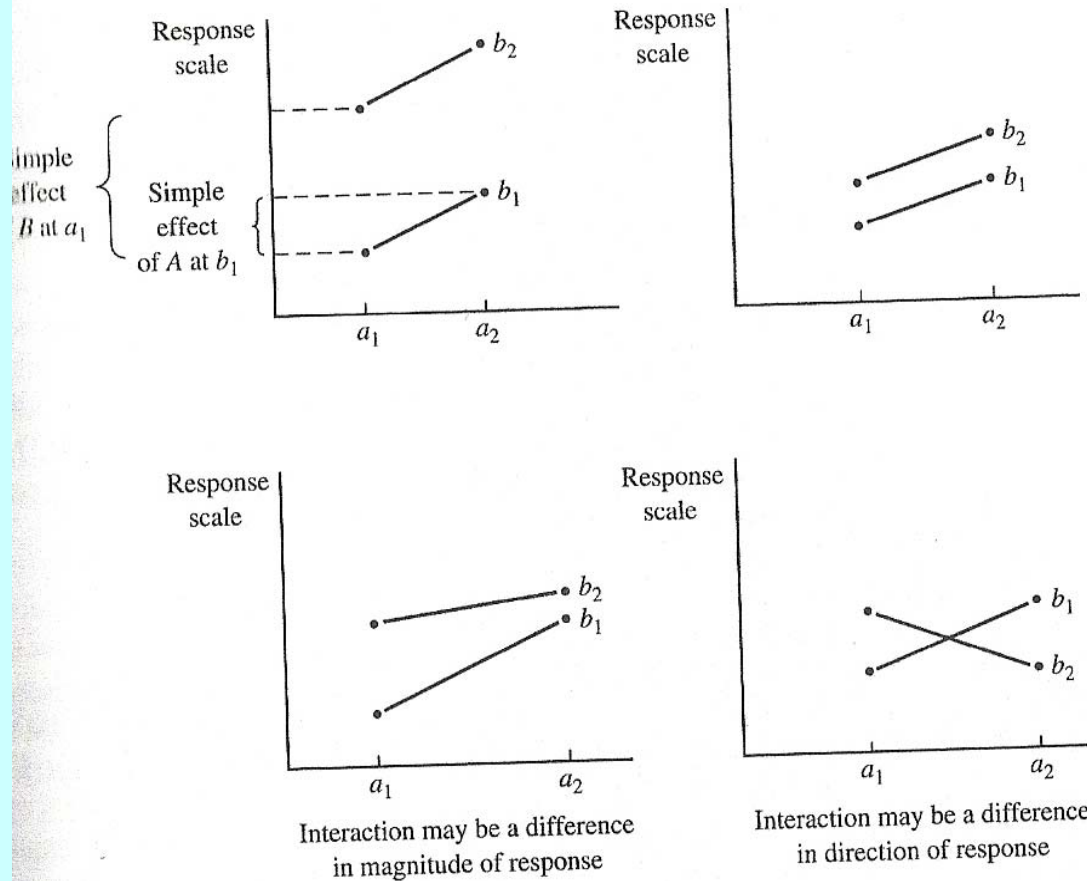


FIGURE 15.1  
Illustration of interaction.

Top Panels

No  
Interaction

Additive  
Responses

Bottom  
Panels

Interaction  
of Different  
Types

# Factorial Treatment Structure

- Very important treatment design structure for where there are 2 **or more** factors at multiple levels
- Each component has a line in the expanded ANOVA table and the df of each is also important. If **additive** the interaction test is not significant whereas if **non additive** then the interaction F test will be significant.
- Note that **main effects** of factors and their **interaction** are now imp. Are the factors additive in effect or not? (ie if not they are interacting). Look at Table of Means and plots like the previous one to assess nature of interaction.
- The main effect of a factor involves its **marginal means** ie. the means averaged over all levels of the other factor. They are really only useful if the factors are not interacting (ie are additive). (**Hidden Replication if no interaction because you are averaging over the other factor**)

# Hidden Replication in Factorials

- 2x3 factorial with 4 replicates. There are 24 total exptl unites
- Each of the 6 treatments has 4 replicates.
- Main effect of A. Each of the 2 means is averaged over the 4 replicates and the 3 levels of factor B for a hidden replication level of 12. ( $12+12=24$ )
- Main Effect of B. Each of the 3 means is averaged over the 4 replicates and the two levels of factor A for a hidden replication level of 8. ( $8+8+8=24$ )

# Analysis of Covariance

- An alternative (or addition) to blocking to increase precision for comparison of treatments when there is an important **auxiliary continuous** variable.
- Can be viewed as a **combination** of a **regression** and an **ANOVA** model.

# Analysis of Covariance

Here I illustrate with a Completely Random Design with a Covariate (x)

$$y_{ij} = \mu + \tau_i + \beta(X_{ij} - \bar{X}) + \varepsilon_{ij}$$

Notes:

- Extends the earlier linear additive model in simple way.
- Could also use with other designs ( R Complete Block)
- Another key point is to note that method assumes  $\beta$  the slope of the line does not change with the treatment.

# Analysis of Covariance: Diagram

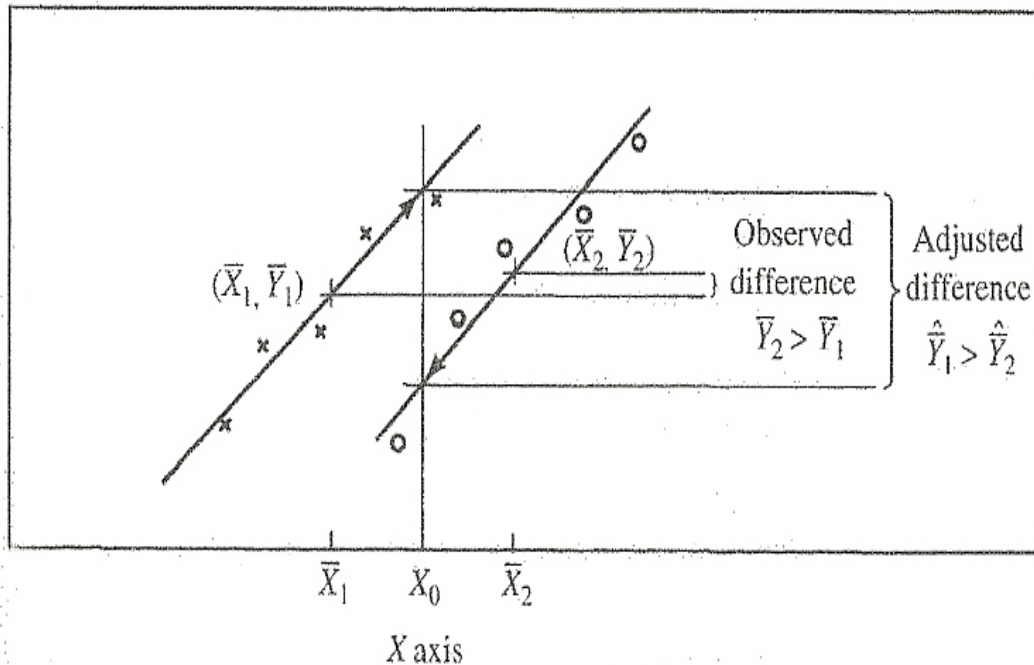
We now illustrate the value of the ANCOVA graphically by considering just 2 treatments and one covariate  $x$ .

In the plot that follows the  $Y$  axis represents the response variable and we consider the two separate regressions (one for each treatment)

Notice the lines are parallel because they are forced to have the same slope ( $\beta$ ).

Observed and adjusted differences in the treatment means are shown.

# Analysis of Covariance: Diagram



Notice how the observed difference in the treat means is confounded by the different values of  $x$ . The adjusted treat means are at a common value of  $x$ .

FIGURE 17.1  
Error control and adjustment of treatment means by covariance.

# ANCOVA: Uses

- To reduce the unexplained variation (Residual Mean Square) in the data and increase the precision of the estimates of model parameters.
- To adjust treatment means to a common value of the covariate  $x$ . (diagram given last slide).
- This method aids in the understanding of the nature of treatment effects
- This method can also be used to understand the relationship of the response variable to the covariate.
- Later we will note that ANCOVA sometimes used when it is not possible to use randomization of treatments as a way of controlling for a potential confounding variable

# ANCOVA: Example 1

- Fish growth study where we house an individual fish in a tank and treatments could be different diets. Replicate tanks used and treatments randomized to the tanks.
- Goal to compare mean growth rates for the different diets.
- Important covariate would be initial size of the fish (length or weight).

# ANCOVA: Example 2

- Haddad Corridor Study also used by Ellen Damschen to study variables (like species richness) related to the plant community.
- One goal to compare mean species richness for the different patch types (Connected, Isolated Winged, Isolated Rectangular ).
- She found that an important covariate was the soil moisture content in each plot.

# ANCOVA: Some Crucial Points

- For ecologists an extremely valuable technique and quite widely used.
- In true experiments it is very valuable as previously stated.
- If treatments are not randomized (ie if not a true expt.) then it is also very valuable and we will come back to this point later in the course.
- Not always emphasized enough by statisticians in methods classes in my opinion.
- Crucial to check if the slope is invariant to the treatment. If so a very powerful technique. If not more complex comparison of regression procedures needed because then the treatment effect varies with  $x$  as well.

# ANCOVA:SAS Code

## C Random Design

```
Proc glm;  
class treat;  
model y = treat x/SS3;  
lsmeans;  
run;
```

## R Complete Block

```
Proc glm;  
class treat block;  
model y = treat block x/SS3;  
lsmeans;  
run;
```

**NOTE:** y is response variable, x is the covariate, Treat is the treatment variable and Block is the block variable. They appear in the Class statement because they are classification variables whereas x is a continuous regression variable. Add a random block; statement if you want blocks to be a random effect.



# Damschen- Haddad Plants Study

## Factor 1(3 levels)

3 Types of Patches (Treatments factor A)

Randomised

## Factor 2 (2 levels)

Edge vs Center of Patch (treatments factor B)

Not randomized.

# Split Plot Design Another Example

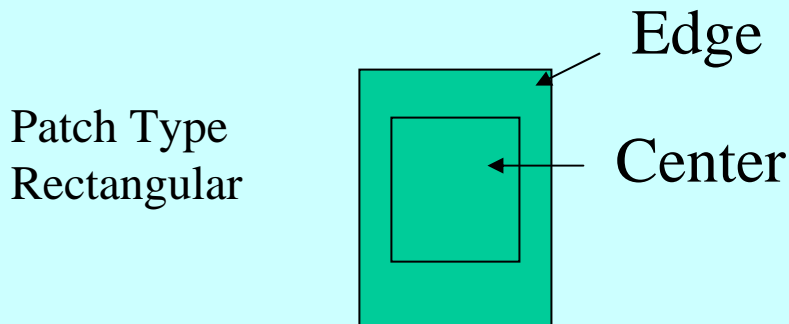
## Damschen- Haddad Plants Study

Types of Patches

Randomised at the level of the whole patch

Edge vs Center of Patch (treatments factor B)

Not randomized but applied to a part of the patch



# Split Plot Design Another Example

## Damschen- Haddad Plants Study

### Whole Plots

3 Types of Patches (Treatments factor A)

Randomised

### Subplots or Split Plots (Within Patches)

Edge vs Center of Patch (treatments factor B)

Not randomized.

Results not presented here but this is what we call a split plot design!! This is a good lead in to this topic!!

# Split Plot Designs

One has a basic design like a randomized block for one treatment factor described earlier for a set of large plots (units) but then one also has another treatment factor applied to smaller subplots within the whole plots.

One is interested in main effects of each factor and their interaction.

There are two different error terms used based on the whole plots and the subplots so the analysis is quite complicated.

# Split Plot Designs

## Linear Additive Model

Randomised blocks ( $\rho$ ) for one treatment factor ( $\alpha$ ) for a set of large plots (units)

One also has another treatment factor ( $\beta$ ) applied to smaller subplots within the whole plots.

$$y_{ijk} = \mu + \rho_i + \alpha_j + \gamma_{ij} + \beta_k + (\alpha\beta)_{jk} + \varepsilon_{ijk}$$

$\gamma_{ij}$  - whole plot residual

$\varepsilon_{ijk}$  - sub plot residual

# Split Plot Design Example ANOVA

6 treatments in a 3x2 factorial design.

5 Blocks

3 Whole Plot Treatments Factor A, 15 Whole Plots

2 Split Plot Treatments 30 Sub Plots (15x2)

# Split Plot Design Example ANOVA

## Whole Plot Only

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
A	2			
Blocks	4			
Residual (a)	8			
<u>Total</u>	<u>15</u>			

5 Blocks, 3 treatments on the whole plots

# Split Plot Design Example

## Full ANOVA Table

**Whole  
Plot  
Part 15**

**Split  
Plot  
Part  
30**

Source	df	SS	MS	F
A	2			
Blocks	4			
Residual (a)	8			
B	1			
AB	2			
Residual (b)	12			
Total	29			

5 Blocks, 6 treatments in a 3x2 factorial design. 3 Whole Plot treats,  
2 Split Plot Treatments (15 Whole Plots, 30 Sub Plots)

# Example ANOVA-Incorrect because it ignores the design structure

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Blocks	4			
A	2			
B	1			
AB	2			
<u>Residual</u>	<u>20</u>			
<u>Total</u>	<u>29</u>			

5 Blocks, 6 treatments in a 3x2 factorial design.

# A Split Plot Design F Tests

## Whole Plots

Factor A Main Effect uses Residual (a) MS as the denominator

## Subplots

Factor B and Interaction AB uses Residual (b) MS as the denominator

**NOTE:** Residual (b) is usually substantially smaller than residual (a) so that the power of the tests for B and AB would be much higher. This should be kept in mind at the design stage.

# Split Plot ANOVA Ch 4

- 2 Reps of whole plot treats, 12 treatments in a 2x6 factorial design. 2 Whole Plot treats (CO<sub>2</sub>), 6 Split Plot Treatments (Nutrients).(4 Whole Plots, 24 Sub Plots in the whole experiment)
- It is very important to realize that the randomization is a two-stage one because of the nested nature of the design. Figure 4.2 page 72 shows the randomization to the 4 whole plots and then to the 6 subplots within each whole plot.
- Table 4.2 p 73 shows the ANOVA Table. It has **some errors** which I correct on the next slide.
- Appendix 4.4 shows the SAS code for a split plot ANOVA.

**NOTE: Earlier Appendices give SAS code for simpler designs CR Design with factorial treatment structure, RCBlock and Augmented RC Block.**

# Corrected Split Plot ANOVA

( Table 4.2 in Scheiner and Gurevitch Book)

<u>Source</u>	<u>df</u>	<u>MS</u>	<u>F</u>	<u>pvalue</u>
CO2	1	130.67	42.96	0.02
Residual (a)	2	3.04		
Nutrient	5	610.09	386.13	0.00
NutrienxCO2	5	7.09	4.49	0.02
<u>Residual (b)</u>	<u>10</u>	<u>1.58</u>		
<u>Total</u>	<u>23</u>			

2 Reps of whole plots, 12 treatments in a 2x6 factorial design. 2 Whole Plot treats, 6 Split Plot Treatments (4 Whole Plots, 24 Sub Plots)

# Split Plot ANOVA (4.2) Results

- Note that the Residual (a) MS is larger than the Residual (b) MS as expected. Here it is about double the size! In many applications there would be an even larger differential between the two residual MS.
- All F tests are significant here. CO<sub>2</sub> used Residual (a) whereas Nutrient and Nutrient x CO<sub>2</sub> used Residual (b)
- Further analysis would involve examining the 2 x 6 table of treatment means and their standard errors. Here as there is interaction between the factors the marginal means are not very helpful.

