

Linear models with random effects

Outline for today

- Random vs fixed effects
- Two-factor example
- Why the calculations are different with random effects
- Problem of unbalanced designs with random effects
- Examples of experiments with random effects
- Linear mixed-effects models
- Example: Estimating repeatability of a measurement
- Assumptions of linear mixed-effects models
- An example violating the assumptions, with solutions

What are fixed effects

Groups are predetermined, of direct interest, repeatable.

For example:

- medical treatments in a clinical trial
- predetermined doses of a toxin
- age groups in a population
- habitat, season, etc.

Any conclusions reached in the study about differences among groups can be applied only to the groups included in the study. The results cannot be generalized to other treatments, habitats, etc. not included in the study.

Example of factorial experiment with fixed effects

Data: Field transplant experiment to investigate how herbivores affect the abundance of the red alga, *Mazzaella parksii* in the intertidal habitat of coastal Washington State using (Harley 2004). I analyzed a subset of treatments:

intertidal zone	herbivory treatment	
	present	absent
low	$n = 16$ plots	$n = 16$ plots
mid	$n = 16$ plots	$n = 16$ plots



$N = 64$ plots in a completely randomized design

ANOVA table for factorial experiment with fixed effects

The denominator of the F statistic for the treatment effect is MS_{residual}

Source	SS	df	MS	F	P
herbivory	1512	1	1512	$1512/14271 = 64.3$	0.01
zone	89	1	89	$89/14271 = 10.5$	0.54
herbivory*zone	2617	1	2617	$2617/14271 = 2.07$	<0.01
residual (error)	14271	60	14271		

64 plots, and as many as 60 degrees of freedom in the error term to test main treatment effect (herbivory). Excellent!

What are random effects

Measurements that come in groups. A group can be

- a family made up of siblings
- a subject measured repeatedly
- a transect of quadrats in a sampling survey
- a plot of plants in a field experiment

Groups are assumed to be randomly sampled from a population of groups.

Therefore, conclusions reached about groups *can* be generalized to the population of groups.

With random effects, the variance among groups is the main quantity of interest, not the specific group attributes.

Example: an experiment with 1 fixed and 1 random effect



Futuyma and Philippi (1987)

Fall cankerworm, *Alsophila pometaria*

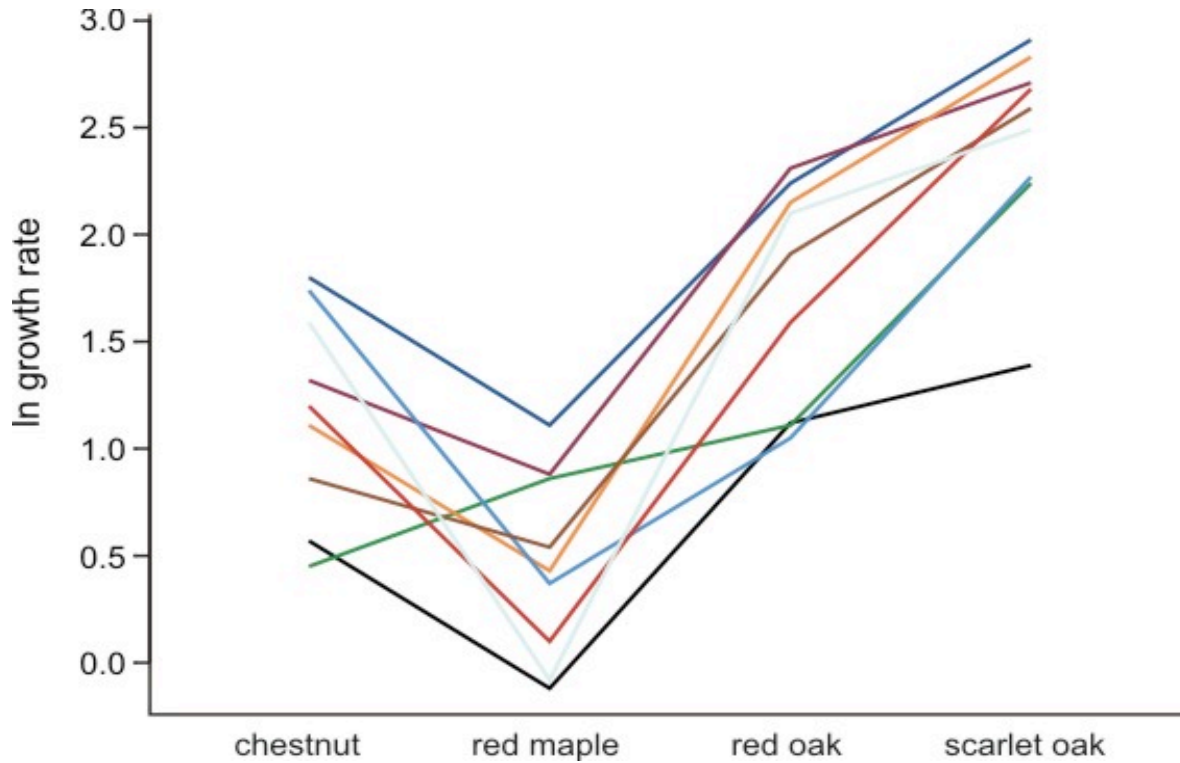
<http://cfs.nrcan.gc.ca/subsite/glfc-sugarbush/alsophila-pometaria-images>

Caterpillars of the fall cankerworm feed on the leaves of hardwood trees. Adult female moths are wingless. Many reproduce clonally, producing only daughters genetically identical to themselves.

Research question: how much genetic variation is present in the moth population for performance on different tree species?

Interaction plot of responses

Design: Sample 9 female moths from a population in NY. Raise larvae from 9 clones on leaves of 4 tree species. Measure growth after 15 days.



Two factors:

Fixed: Tree species

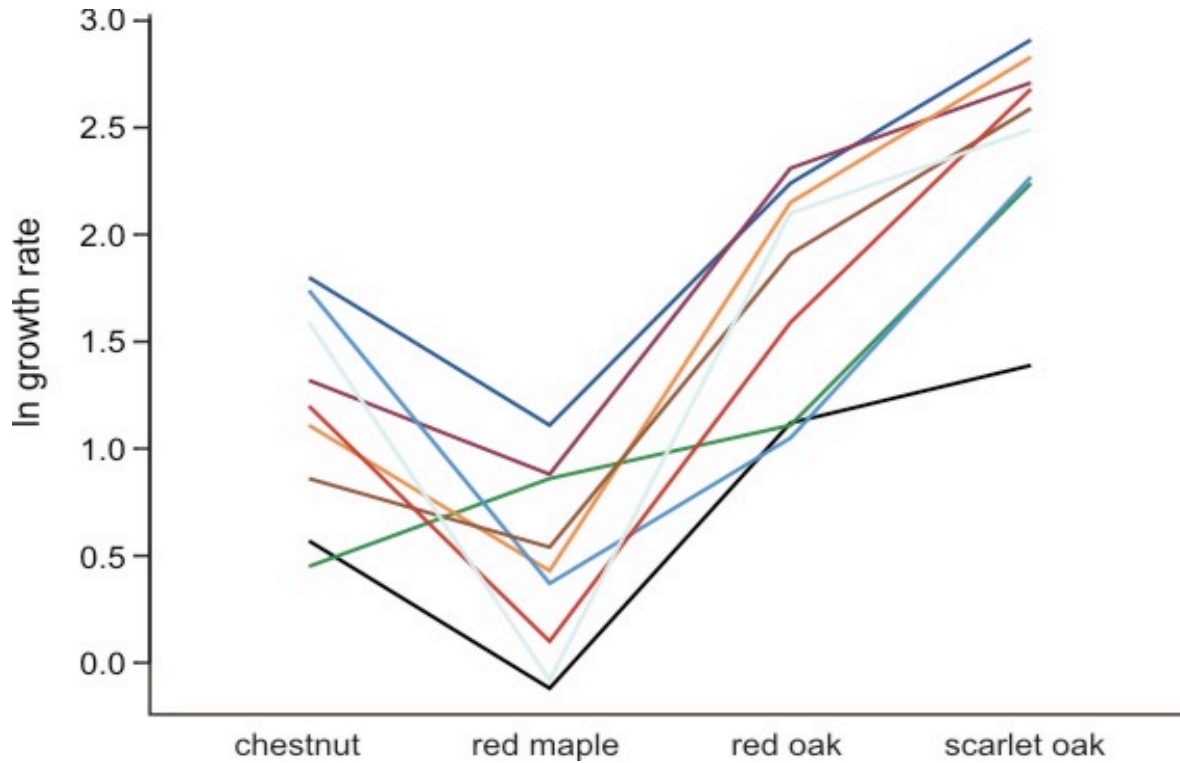
Random: Clone

Growth rates of caterpillars from nine families (clones) raised on four trees species.

$N = 326$ caterpillars total.

Interaction plot of responses

Design: Sample 9 female moths from a population in NY. Raise larvae from 9 clones on leaves of 4 tree species. Measure growth after 15 days.



Two factors:

Fixed:

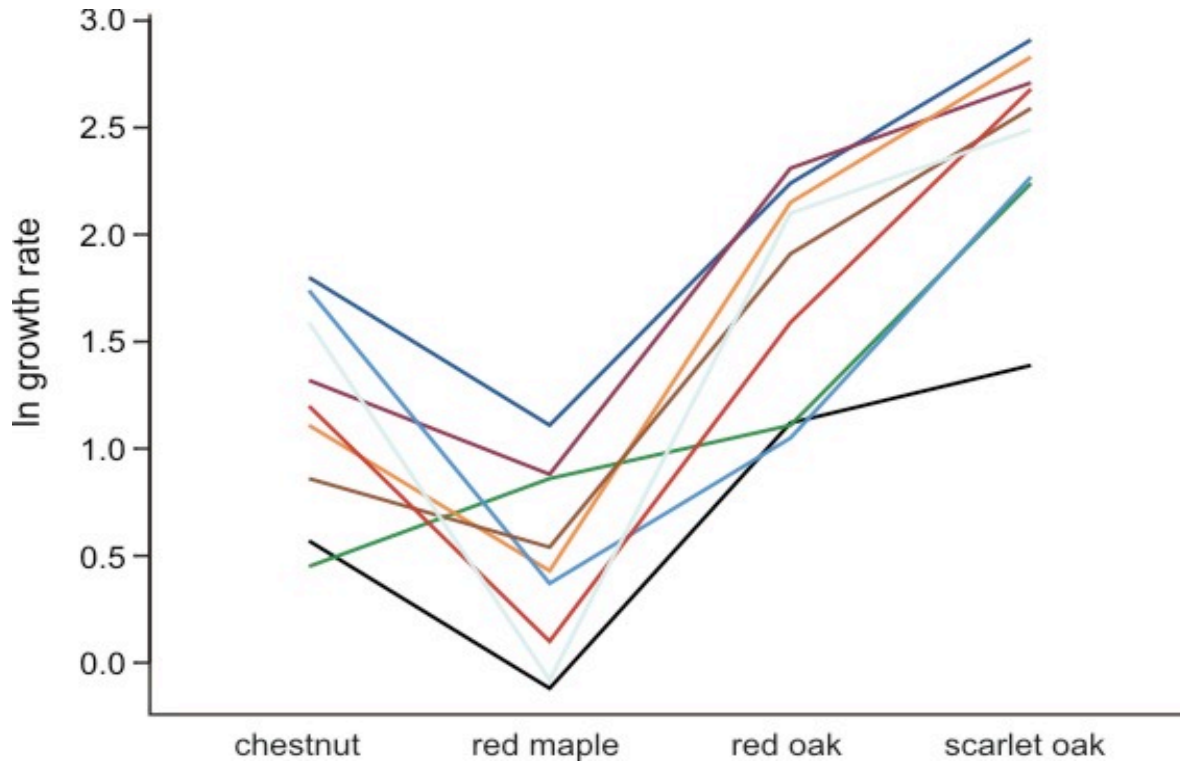
Random:

Growth rates of caterpillars from nine families (clones) raised on four trees species.

$N = 326$ caterpillars total.

Interaction plot of responses

Design: Sample 9 female moths from a population in NY. Raise larvae from 9 clones on leaves of 4 tree species. Measure growth after 15 days.



Two factors:

Fixed: Tree species

Random: Clone

Growth rates of caterpillars from nine families (clones) raised on four trees species.

$N = 326$ caterpillars total.

Why random effects must be analyzed differently

F-statistics are calculated differently than with fixed effects

The denominator of the *F* statistic for the fixed effect is not MS_{residual}

Source	SS	df	MS	<i>F</i>	<i>P</i>
tree	167.7	3	55.9	$55.9/0.9 = 64.3$	<0.001
clone	35.3	8	4.4		
clone*tree	21.0	24	0.9		
residual	122.4	290	0.4		

290 df for error and they can't be used to test treatment effects!

This is because caterpillars from the same clone are not independent.

There are only 9 clones, and it would be pseudoreplication to base test of treatment effect on the number of caterpillars.

Correctly dealing with random effects avoids pseudoreplication

What the *F*-statistic for treatment is made of

F is a ratio of Mean Squares. The Mean Squares are chosen so that any variance due to treatment stands out from “other variance”

$$F = \frac{\text{(variance due to treatment) + (other variance)}}{\text{(other variance)}}$$

Fixed effects:

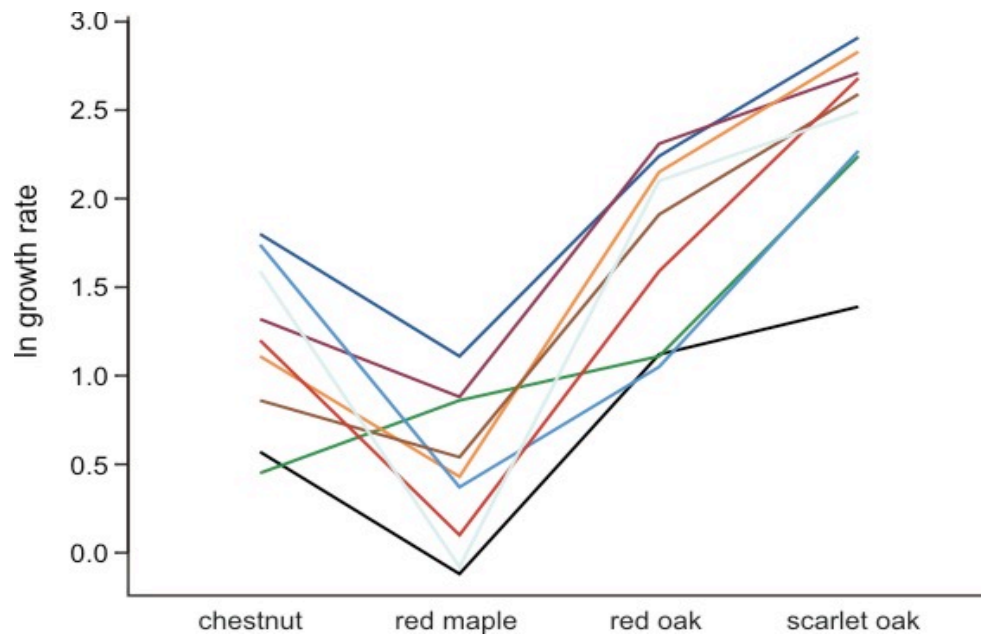
$$MS_{\text{treatment}} = \text{variance treatment} + \text{variance sampling error}$$

$$MS_{\text{residual}} = \text{variance sampling error}$$

In a fixed effects model, the **residual error** variance (representing sampling error) makes up all the “variance due to other things”. It is the *only* source of randomness in the design. All else is fixed.

Why the F -statistic is calculated differently with random effects

The presence of a random factor adds another layer of variance to estimates of treatment effects, arising from differences between clones in how they respond to tree species (interactions).



Expected variation among treatments changes depending on which clones happened to be randomly sampled by the researchers.

F-statistic for treatment when there are random effects

$$F = \frac{(\text{variance treatment}) + (\textit{other variance})}{(\textit{other variance})}$$

When a random effect is present:

$$MS_{\text{treatment}} = \text{variance treatment} + \text{variance interaction} + \text{variance sampling error}$$

$$MS_{\text{residual}} = \text{variance sampling error}$$

$$MS_{\text{interaction}} = \text{variance interaction} + \text{variance sampling error}$$

So to test treatment effect we use

$$F = MS_{\text{treatment}} / MS_{\text{interaction}}$$

Caution when analyzing data on the computer

Most statistical packages assume that all factors are fixed unless you instruct it otherwise.

Designating factors as random takes extra work and probably a read of the manual.

In R, `lm` assumes that all effects are fixed.

For random effects, use `lme` instead (part of the `nlme` package).

More reasons why random effects are special

1. Means of random groups (e.g., clones) are not of interest by themselves. Only the variance among random groups and variance associated with interactions are of interest (variance components). This was the purpose of the Futuyma and Philippi experiment: to estimate genetic variance in the population. So F -test for random effects are not very useful (`lme` in R won't calculate them).
2. When a design including random effects is unbalanced, the F statistics are not F -distributed. Standard ANOVA calculations with random effects do not work when sample sizes differ among treatment levels. Sorry!
3. Mixed-effects models is a flexible and accurate method for analyzing data with random effects.

How to know when you have random effects in your study

You have random effects:

- whenever your sampling design is **nested** (quadrats within transects; transects within woodlots; woodlots within districts).
- whenever your replicates are **grouped** spatially or temporally (i.e., in blocks, which are typically analyzed as random effects).
- whenever you **divide up plots**, and apply separate treatments to subplots.
- whenever you take measurements on **related individuals**.
- whenever you measure subjects or other **sampling units repeatedly**.

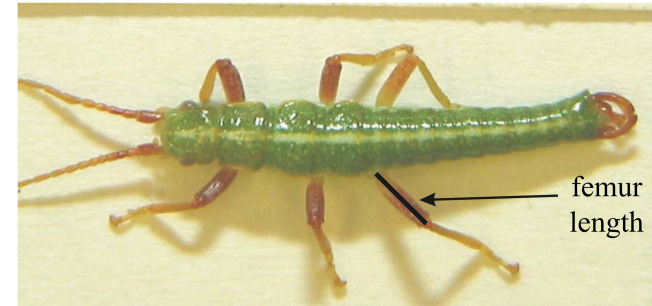
[break]

Attributes of linear mixed-effects models

- Linear models that include both fixed and random effects.
- The model is split into fixed and random parts:
 - Fixed effects influence mean of the response variable Y .
 - Random effects influence the variance of Y .
- There is a different error variance for each level of grouping.
- Estimation and testing is based on restricted maximum likelihood, which can handle *unequal* sample size.
- P -values for fixed effects are conservative when design unbalanced.
- Implemented in the `nlme` package in R.

Example 1: Study of “repeatability” (simple nested design)

The walking stick, *Timema cristinae*, is a wingless herbivorous insect on plants in chaparral habitats of California. Nosil and Crespi (2006) measured individuals using digital photographs. To evaluate measurement repeatability they took two separate photographs of each specimen. After measuring traits on one set of photographs, they repeated the measurements on the second set.



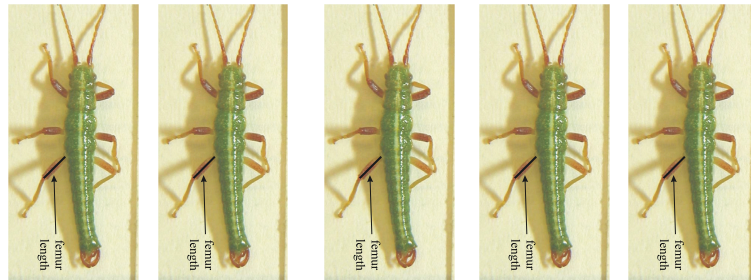
Factor:

Random: Individual

The pair of femur length measurements of 25 walking stick insects.

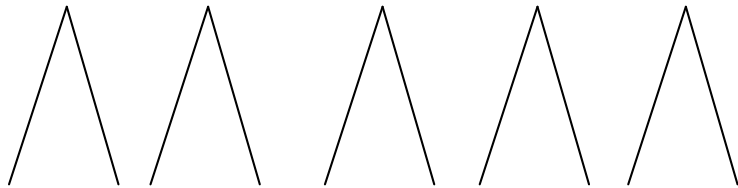
Example R analysis: Study of “repeatability” (simple nested design)

It can be helpful to draw a sketch of the design



individual bug

.....



1 2 1 2 1 2 1 2 1 2

Measurements “within” individuals

ANOVA table of femur length measurements

Source	SS	df	MS	<i>F</i>	<i>P</i>
group (insects)	0.0059	24	0.0025	0.0025/0.0004 = 6.902	< 0.001
error	0.0089	25	0.0004		

Significant result: what does this mean?

Despite measurement error, detectable difference among insects exist.

Repeatability

Single factor ANOVA with random effects: 2 levels of random variation.

- 1) within groups (individual insect) *also in fixed effect model*
- 2) between groups (different insects) *not random in f. e. model*

Variance components

1) assumed to be the same for all groups: $\sigma^2_{\text{within}} = MS_{\text{error}}$

2) assumed to vary according to a normal distribution in the population:

$$\sigma^2_{\text{among}} = ((MS_{\text{groups}} - MS_{\text{error}}) / n)$$

$$\text{Repeatability} = \sigma^2_{\text{among}} / (\sigma^2_{\text{among}} + \sigma^2_{\text{within}})$$

Example R analysis: Study of “repeatability” (simple nested design)

```
library(nlme)
```

```
z <- lme(femurlength ~ 1, random= ~1 | individual)
```

```
VarCorr(z)
```

	Variance	StdDev	
(Intercept)	0.00105..	0.03246..	# var among individuals (σ_a^2)
Residual	0.00035..	0.01886..	# var within individuals (σ_a^2)

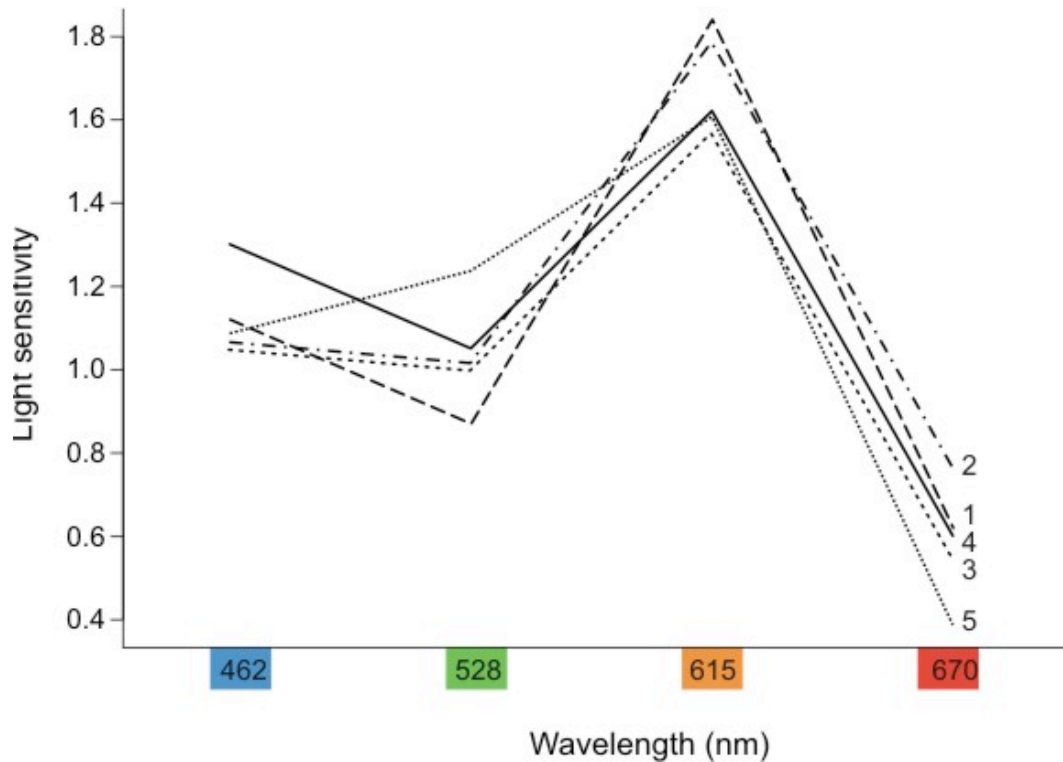
Use these quantities to calculate repeatability

```
estimate = 0.00105.. / (0.00105.. + 0.00035.. )
```

```
= 0.75
```

Example 2: “Subjects by treatment design”

Cronly-Dillon and Muntz (1965) used the optomotor response to measure color vision in the goldfish. Each fish was tested at different wavelength in random order. A large value indicates that the fish can detect a low light intensity (= high sensitivity).



Factors:

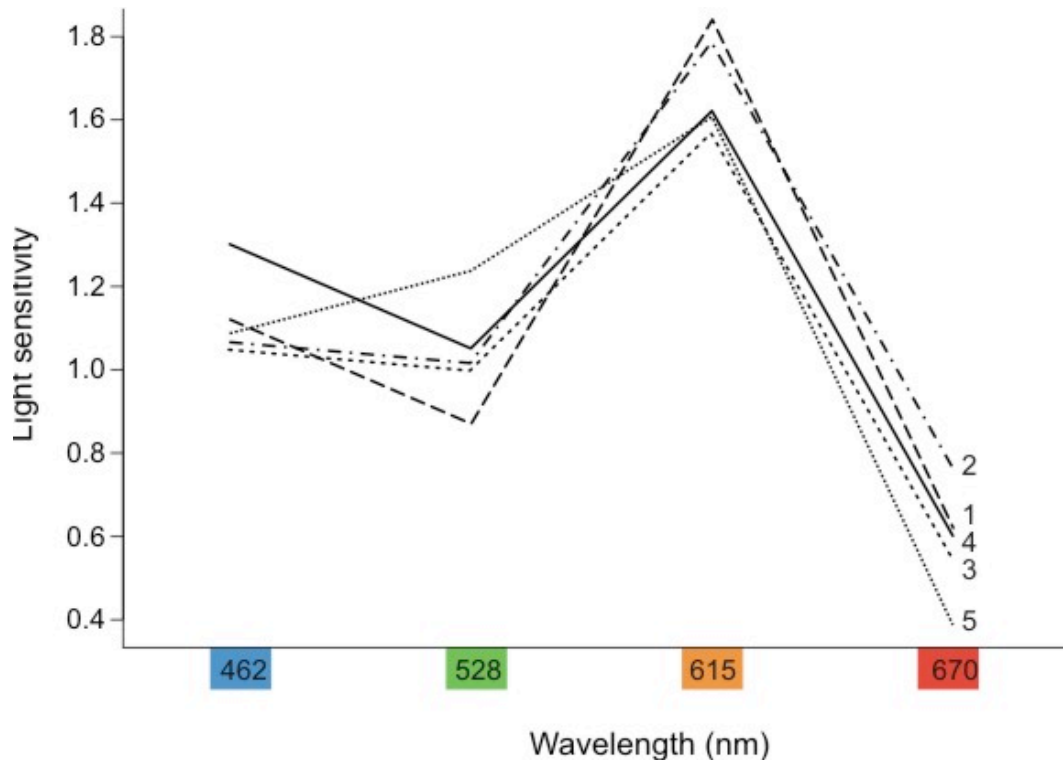
Fixed:

Random:

Light sensitivity of 5 goldfish to specific wavelengths of light.

Example 2: “Subjects by treatment design”

Cronly-Dillon and Muntz (1965) used the optomotor response to measure color vision in the goldfish. Each fish was tested at different wavelength in random order. A large value indicates that the fish can detect a low light intensity (= high sensitivity).



Factors:

Fixed: Wavelength (repeated measure)

Random: Fish

Light sensitivity of 5 goldfish to specific wavelengths of light.

Example 3: Split plot design

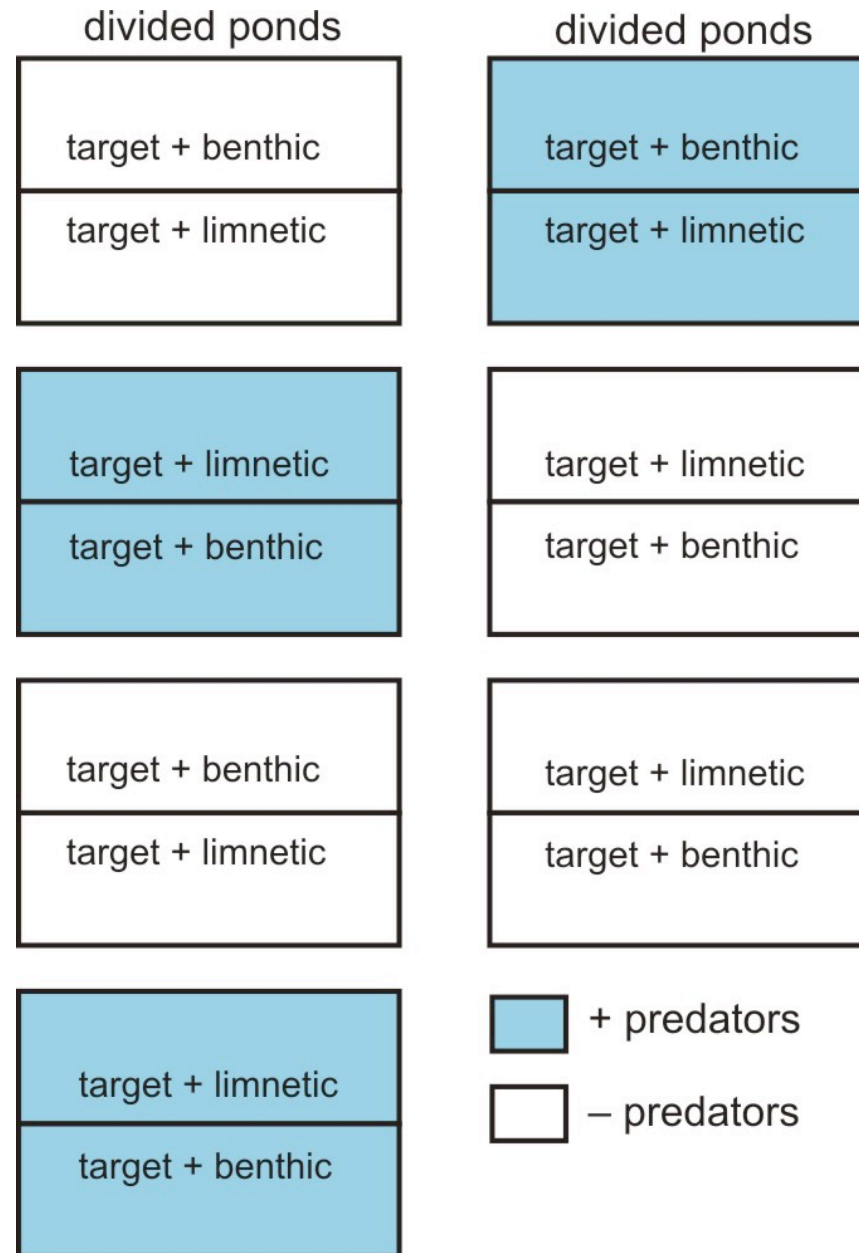


Factors:

Fixed:

Fixed:

Random:



Example 3: Split plot design

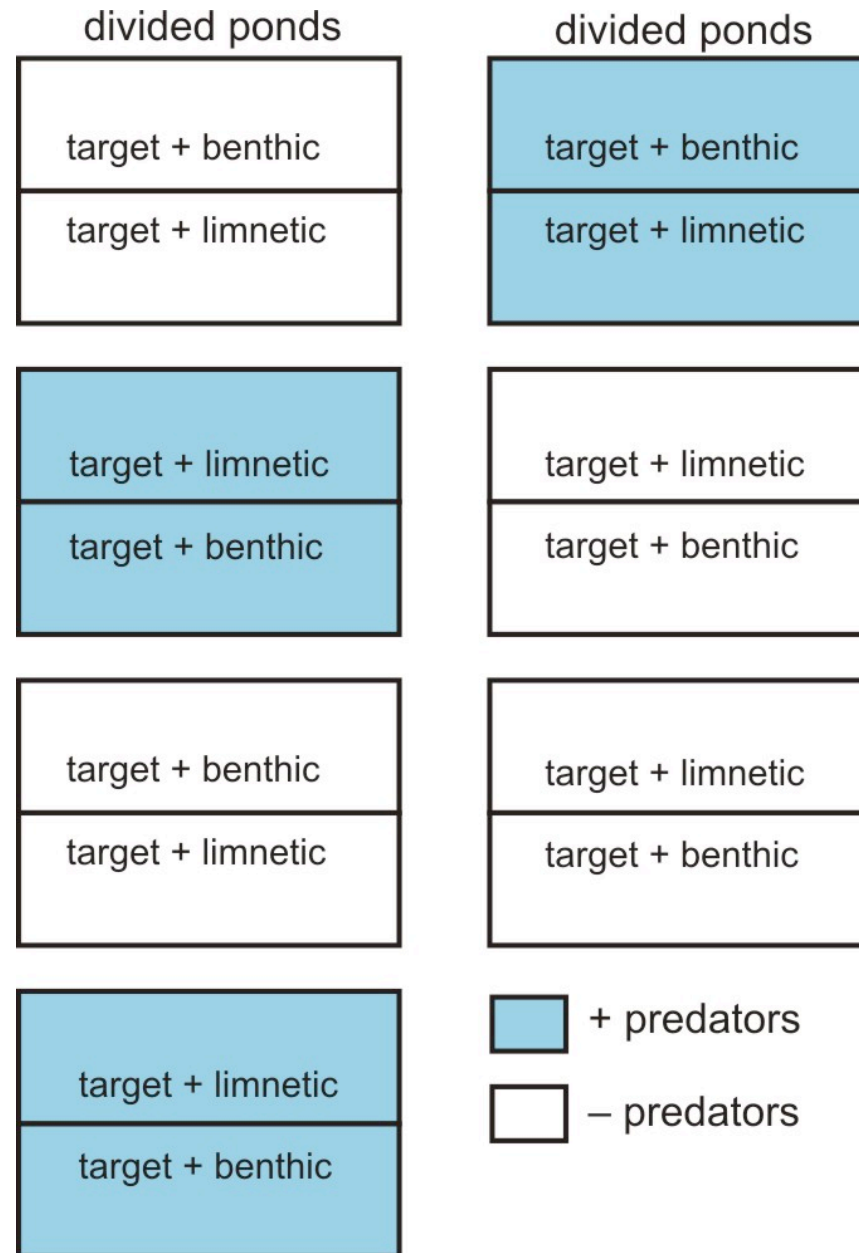


Factors:

Fixed: Competition treatment

Fixed: Predation treatment

Random: Pond



Assumptions of linear mixed-effects models

- Variation within groups follows a normal distribution with equal variance among groups.
- Groups are randomly sampled from “population” of groups.
- Group means follow a normal distribution.
- Measurements within groups are independent.
- No carry-over between repeated measurements on the same subject.
- “Sphericity”: the variance of the differences between values of the response variable is the same for all pairs of treatments (see below).

Example 4: “Subjects by trials design”

Ecology, 65(6), 1984, pp. 1780–1786
 © 1984 by the Ecological Society of America

GRANIVORY IN A DESERT ECOSYSTEM: EXPERIMENTAL EVIDENCE FOR INDIRECT FACILITATION OF ANTS BY RODENTS¹

D. W. DAVIDSON
 Department of Biology, University of Utah, Salt Lake City, Utah 84112 USA

AND

R. S. INOUE² AND J. H. BROWN
 Department of Ecology and Evolutionary Biology, University of Arizona,
 Tucson, Arizona 85721 USA

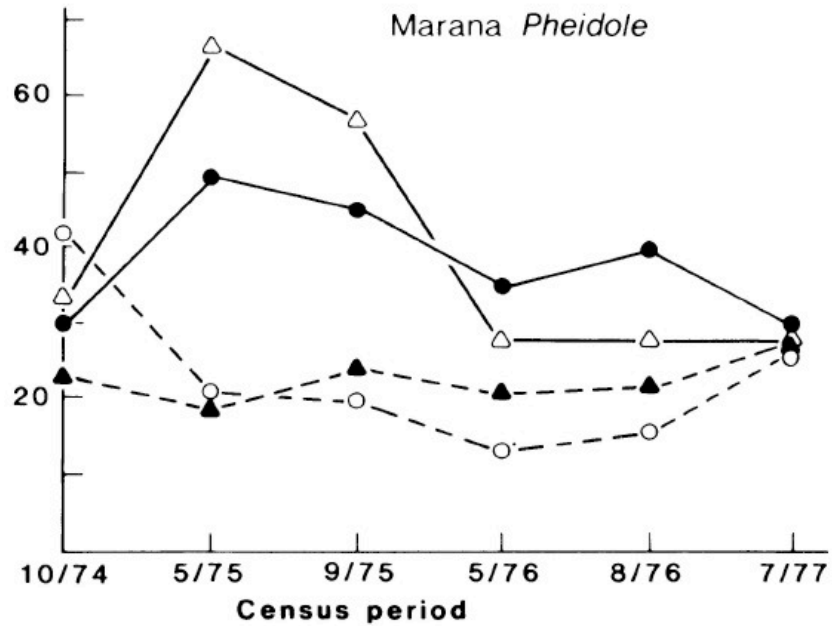


FIG. 3. Changes in density of *Pheidole* spp. (including *P. rophila tucsonica*, *P. sitarches*, and *P. gilvescens*) on two lent removal plots (—) and two control plots (— —) at arana, Arizona over a 2³/₄-yr period.

http://www.tightloop.com/ants/showgenus.php?genus_id=20

Factors:

Fixed:

Fixed:

Random:

Example 4: “Subjects by trials design”

Ecology, 65(6), 1984, pp. 1780–1786
 © 1984 by the Ecological Society of America

GRANIVORY IN A DESERT ECOSYSTEM: EXPERIMENTAL EVIDENCE FOR INDIRECT FACILITATION OF ANTS BY RODENTS¹

D. W. DAVIDSON
 Department of Biology, University of Utah, Salt Lake City, Utah 84112 USA

AND

R. S. INOUE² AND J. H. BROWN
 Department of Ecology and Evolutionary Biology, University of Arizona,
 Tucson, Arizona 85721 USA

Marana *Pheidole*

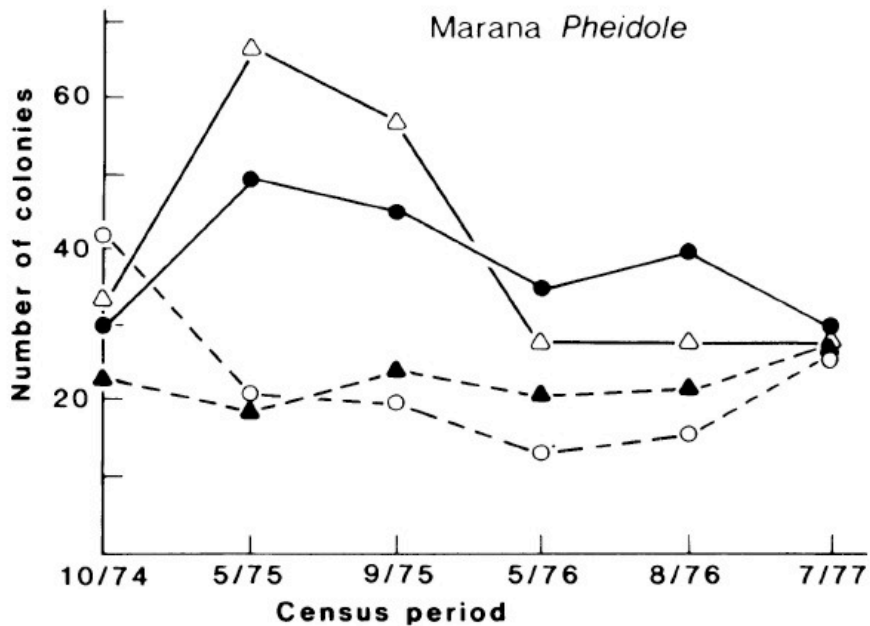


FIG. 3. Changes in density of *Pheidole* spp. (including *P. xerophila tucsonica*, *P. sitarches*, and *P. gilvoscens*) on two rodent removal plots (—) and two control plots (— —) at Marana, Arizona over a 2³/₄-yr period.



http://www.tightloop.com/ants/showgenus.php?genus_id=20

Factors:

Fixed: Rodent treatment (fixed)

Fixed: Time (repeated measure)*

Random: Plot

*errors are correlated...

An example violating the assumptions of linear mixed models

How to analyze these results?

Tempting to use linear mixed-effects model

Fixed part:

```
fixed=ncolonies ~ treatment  
      + time + treatment*time
```

Random part:

```
random= ~1 | plot
```

Problem: “time” violates the sphericity assumption. Measurements close together in time are more highly correlated than values farther apart in time.

An example violating the assumptions of linear mixed models

Time can't be considered a valid factor in repeated measures studies

How to analyze these results?

1. Separate two-sample t-test at each date
2. MANOVA (not enough replicates)
3. Profile analysis
4. Single two-sample t -test on a summary statistic

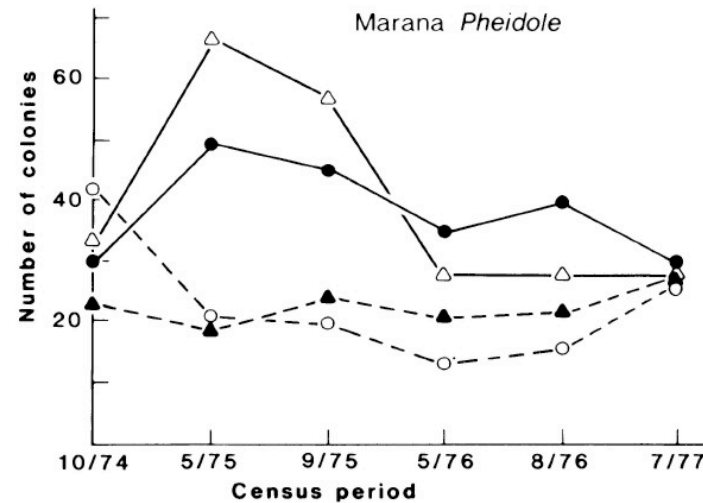
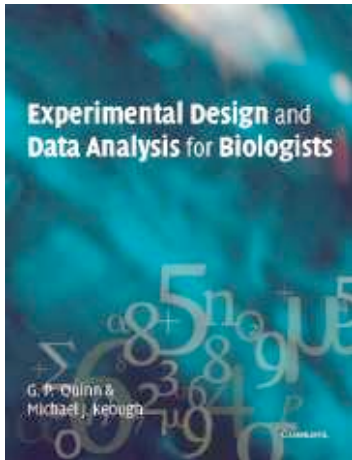


FIG. 3. Changes in density of *Pheidole* spp. (including *P. xerophila tucsonica*, *P. sitarches*, and *P. gilvescens*) on two rodent removal plots (—) and two control plots (---) at Marana, Arizona over a 2¾-yr period.

Where to get further advice

I have found the following book to be very useful in understanding design and assumptions of mixed-effects models

though it won't help you with modeling using maximum likelihood.



Quinn & Keough 2002

Experimental Design and Data Analysis for Biologists

Pinheiro and Bates (2000). Mixed-effects models in S and S-PLUS

(online book at UBC library – see link on textbook tab.) At least the first chapter is understandable

The R book (Crawley 2007) has good advice on analyzing in R

Discussion paper for next week:

Murtaugh (2007) Simplicity and complexity in data analysis.
Ecology 88: 56–62.

Presenters: [your name] and [somebody else]

Moderators: [...] and [...]