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

Predetermined categories of a variable, of direct interest, repeatable.

For example:

- medical treatments in a clinical trial
- predetermined doses of a toxin
- diet or fertilization treatments
- age groups in a population
- habitat, season

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

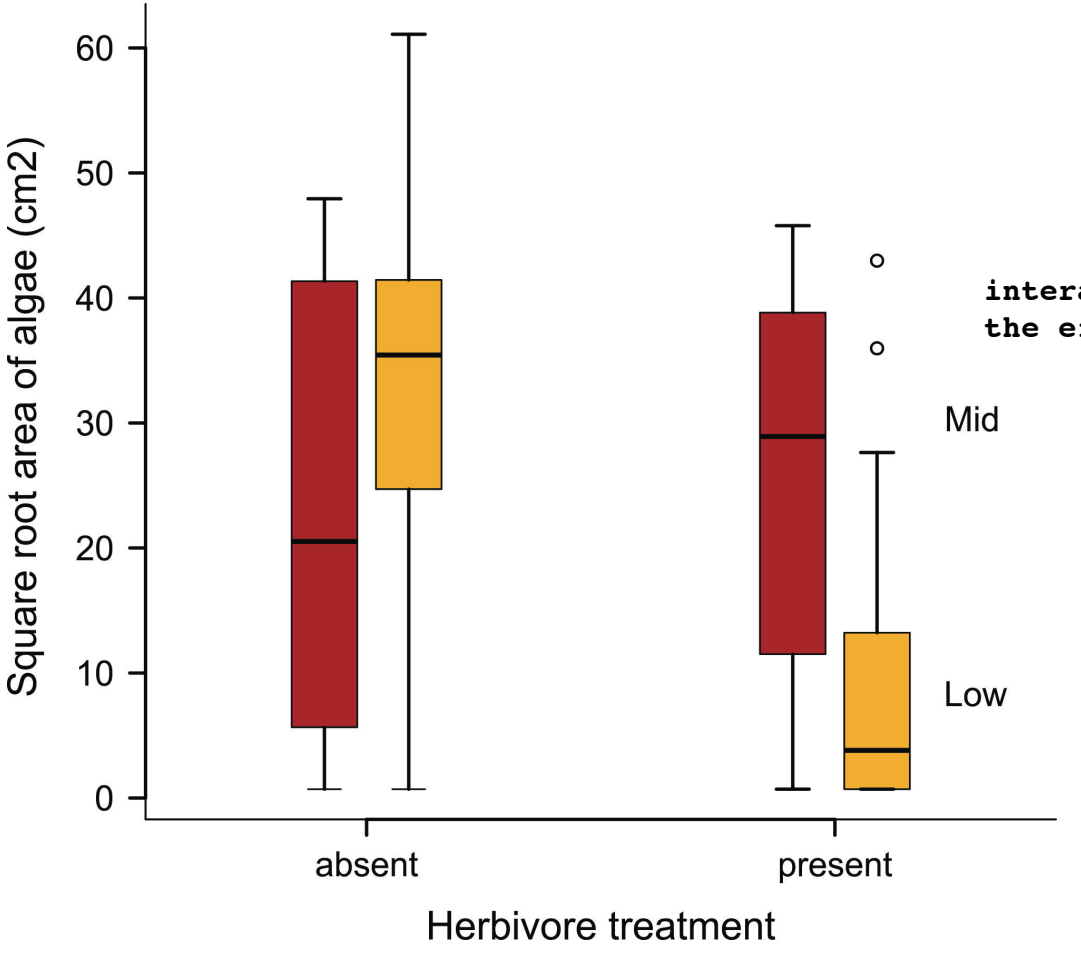
Field transplant experiment to investigate how herbivores affect the abundance of the red alga, *Mazzaella parksii* in the intertidal habitat of coastal Washington State using (Harley 2004). I analyzed a subset of treatments:

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

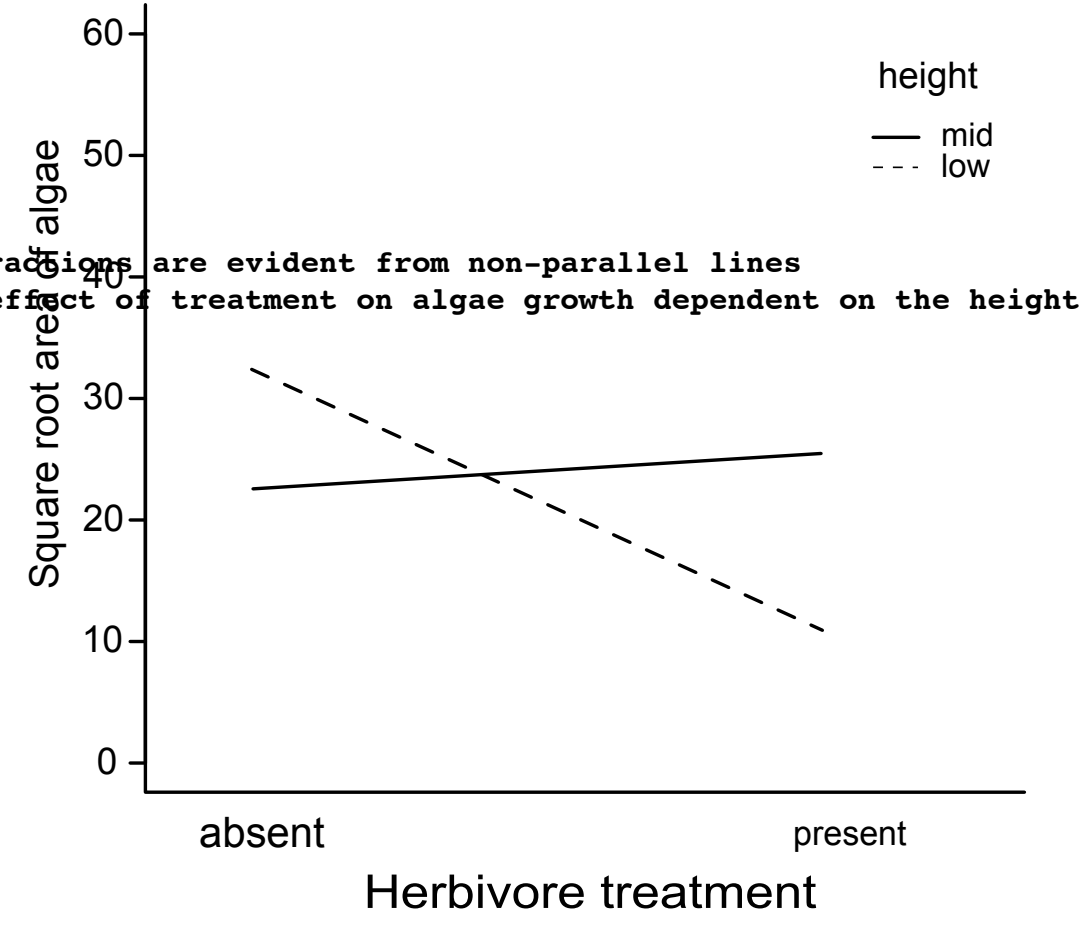
N = 64 plots in a completely randomized design

Factorial experiment with fixed effects

Data: surface area of the red alga, *Mazzaella parksii* in plots in both zones.



interactions are evident from non-parallel lines
the effect of treatment on algae growth dependent on the height



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	1512/238= 6.36	0.01
zone	89	1	89	89/238= 0.37	0.54
herbivory*zone	2617	1	2617	2617/238= 11.0	<0.01
residual (error)	14271	60	238		

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

What are random effects

Randomly sampled categories of a variable, representing groups of measurements or units. For example:

- families made up of siblings
- subjects measured repeatedly (“repeated measures”)
- transects of quadrats in a sampling survey
- field plots of plants
- environment chambers containing aquaria

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.

What are random effects

In some cases the random effects are a nuisance, of no interest themselves.

- field plots
- environment chambers containing aquaria

In other cases, measuring the variance associated with different levels of random groupings is a major point of the study.

- families made up of siblings
- subjects measured repeatedly (“repeated measures”)
- transects of quadrats in a sampling survey

In either case, random effects must be incorporated into the model, because units within groups are not independent (e.g, repeated measures).

Modeling random effects explicitly avoids pseudoreplication.

Example: a factorial 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

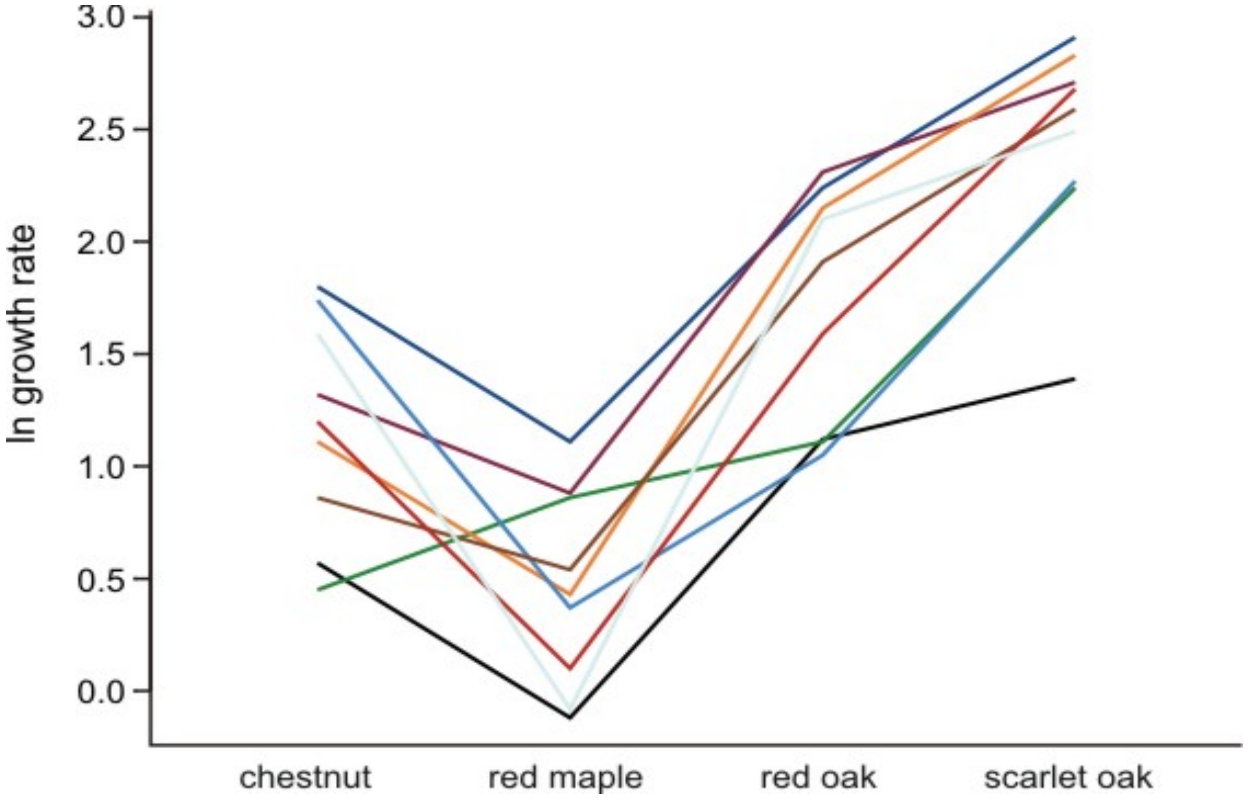
Caterpillars of the fall cankerworm feed on the leaves of hardwood trees. Adult female moths are wingless. Many reproduce clonally, producing only daughters genetically identical to themselves.

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, crossed: **Tree species** (Fixed), ***Clone*** (Random)



Mean growth rates of caterpillars from nine families (clones) raised on four tree species.

N = 326 caterpillars total.

Why random effects must be analyzed differently

F-statistics are calculated differently than with fixed effects.

E.g., the denominator of *F* for the test of the fixed effect is not $MS_{residual}$

Source	SS	df	MS	*F*	*P*
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.

(Always report the df with *F*-statistic to prove you used the correct MS)

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 against the other sources of variance.

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

Fixed effects:

$MS_{treatment}$ = variation due to treatment + variance due to sampling error

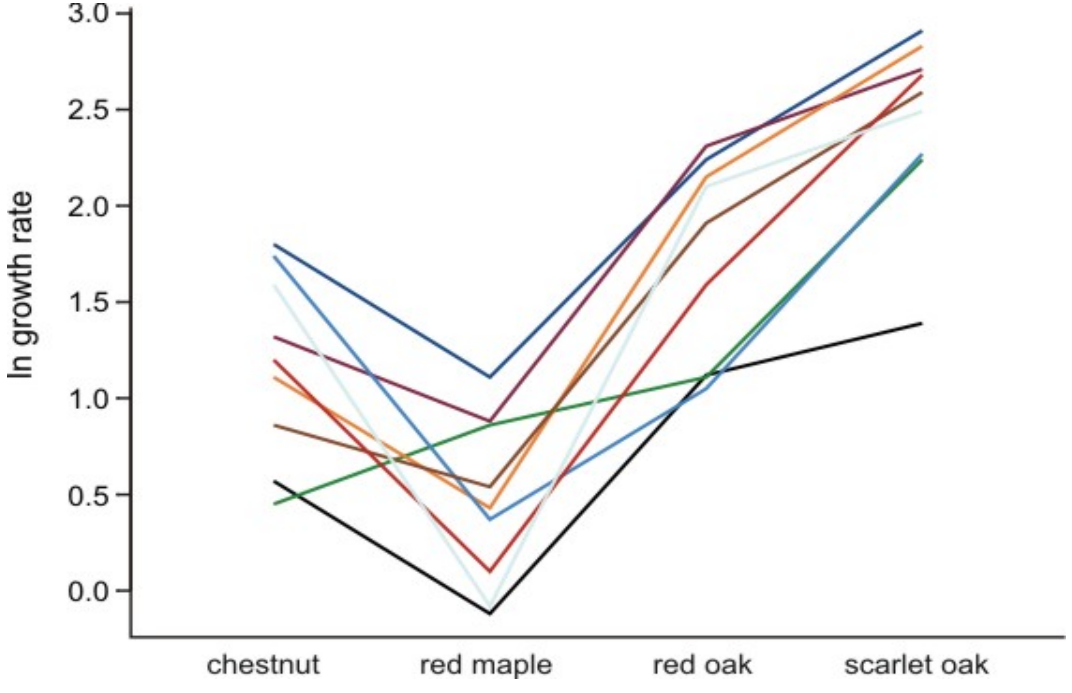
$MS_{residual}$ = variance due to sampling error

In a fixed effects model, the residual error variance (representing sampling error) makes up all the "other variance". 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 source of random variation when estimating treatment effects, arising from interactions (e.g., differences between clones in how they respond to tree species).

Observed differences among treatment means now also depend on which clones happened to be randomly sampled by the researchers.



F-statistic for treatment when there are random effects

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

When a random effect is present:

$MS_{treatment}$ = variation due to treatment + variance due to interaction + variance due to sampling error

$MS_{residual}$ = variance due to sampling error

$MS_{interaction}$ = variance due to interaction + variance due to sampling error

So to test treatment effect we use

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

Caution when analyzing data on the computer

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

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

In R, `lm` assumes that all effects are fixed. Do not use if you have random effects.

Use `lme` (in the `nlme` package) or `lmer` (in the `lme4` package) to analyze models containing random effects. They model the variance structure explicitly, and use restricted maximum likelihood (REML) to obtain unbiased estimates of effects and test hypotheses.

More reasons why random effects are special

1. When a design including random effects is unbalanced, the standard *F* statistics as calculated above are not *F*-distributed. Standard ANOVA table calculations don't work with unequal sample sizes. Sorry!
2. Unlike fixed groups, the means of the random groups (e.g., clones) are not of direct interest. `lme` in R won't carry out an *F* test of differences among means for random effects. Instead, estimates of variance among random groups, and of variance associated with interactions, are of interest (*variance components*). The purpose of the Futuyma and Philippi experiment was to *estimate* variance components.
3. With unbalanced designs, the *F*-statistics and degrees of freedom for fixed effects in mixed models are approximations. `lmer` won't give *P*-values at all, but `lmerTest` will do so.

How to know when you have random effects in your study

You have random effects:

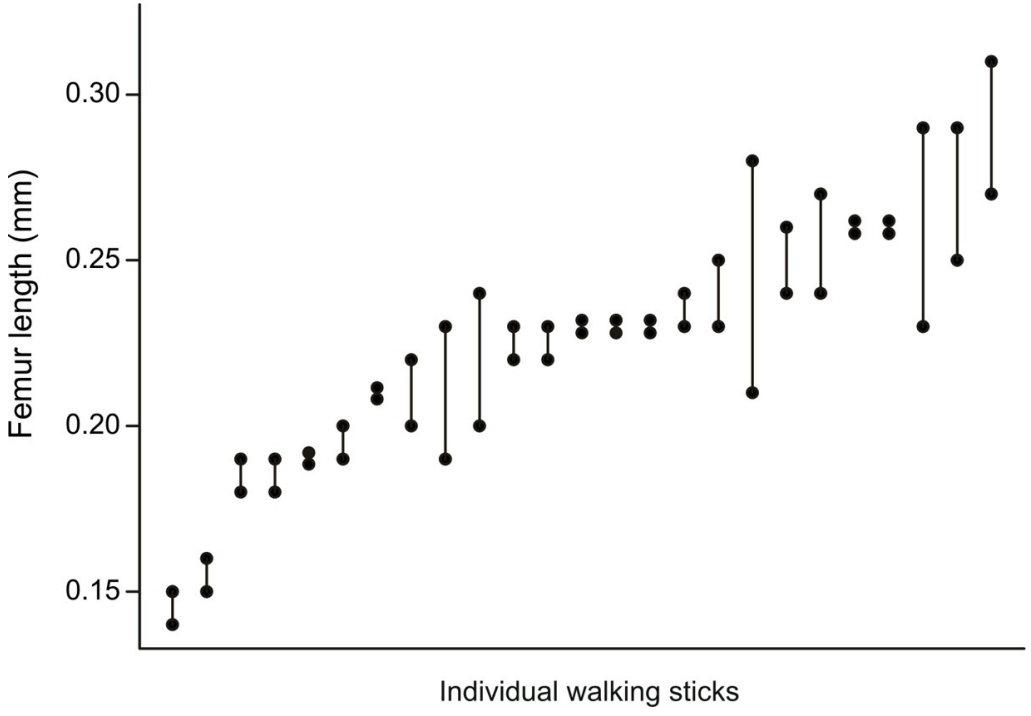
- whenever your sampling design is nested:
  quadrats within transects; transects within woodlots; woodlots within districts.
- whenever your replicates are grouped spatially or temporally
  i.e., in *blocks*, which are typically analyzed as random effects.
- whenever you divide up plots (families, clones,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

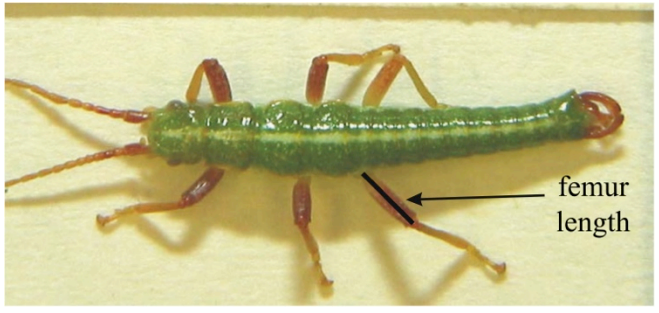
Attributes of linear mixed-effects models

- Linear models that include both fixed and random effects.
- In R, the formula specifying the model is split into fixed and random parts.
- There is a different error variance for each source.
- Estimation and testing are based on restricted maximum likelihood, which can handle unequal sample size.
- *P*-values for fixed effects are conservative when designs are unbalanced.
- Implemented in the `nlme` and `lme4` packages in R.

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

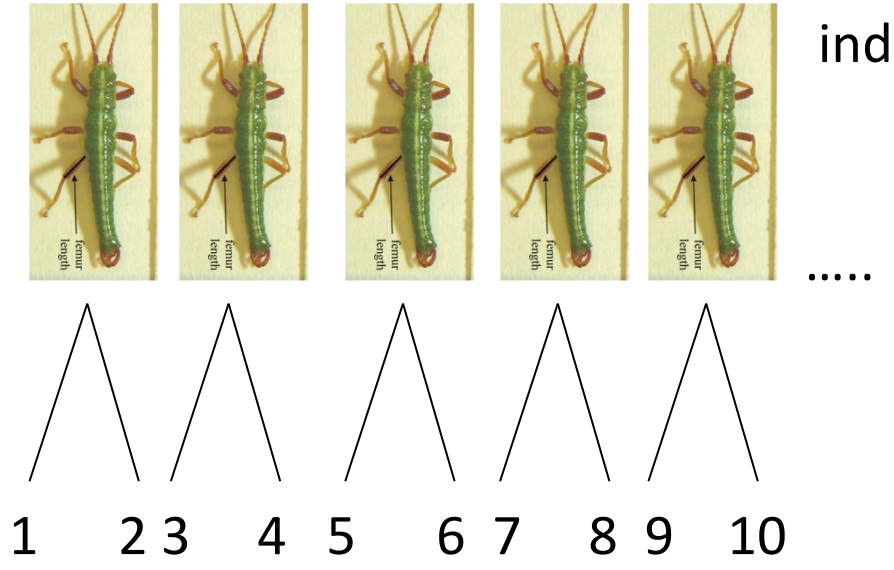
The walking stick, *Timema cristinae*, is a wingless herbivorous insect on plants in chaparral habitats of California. Nosil and Crespi (2006) measured individuals using digital photographs. To evaluate measurement repeatability they took two separate photographs of each specimen. After measuring traits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





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

It can be helpful to draw a sketch of the design



The individuals are the random groups in this study, with two repeated measurements per group.

Example 1: Study of "repeatability" (simple nested design)

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

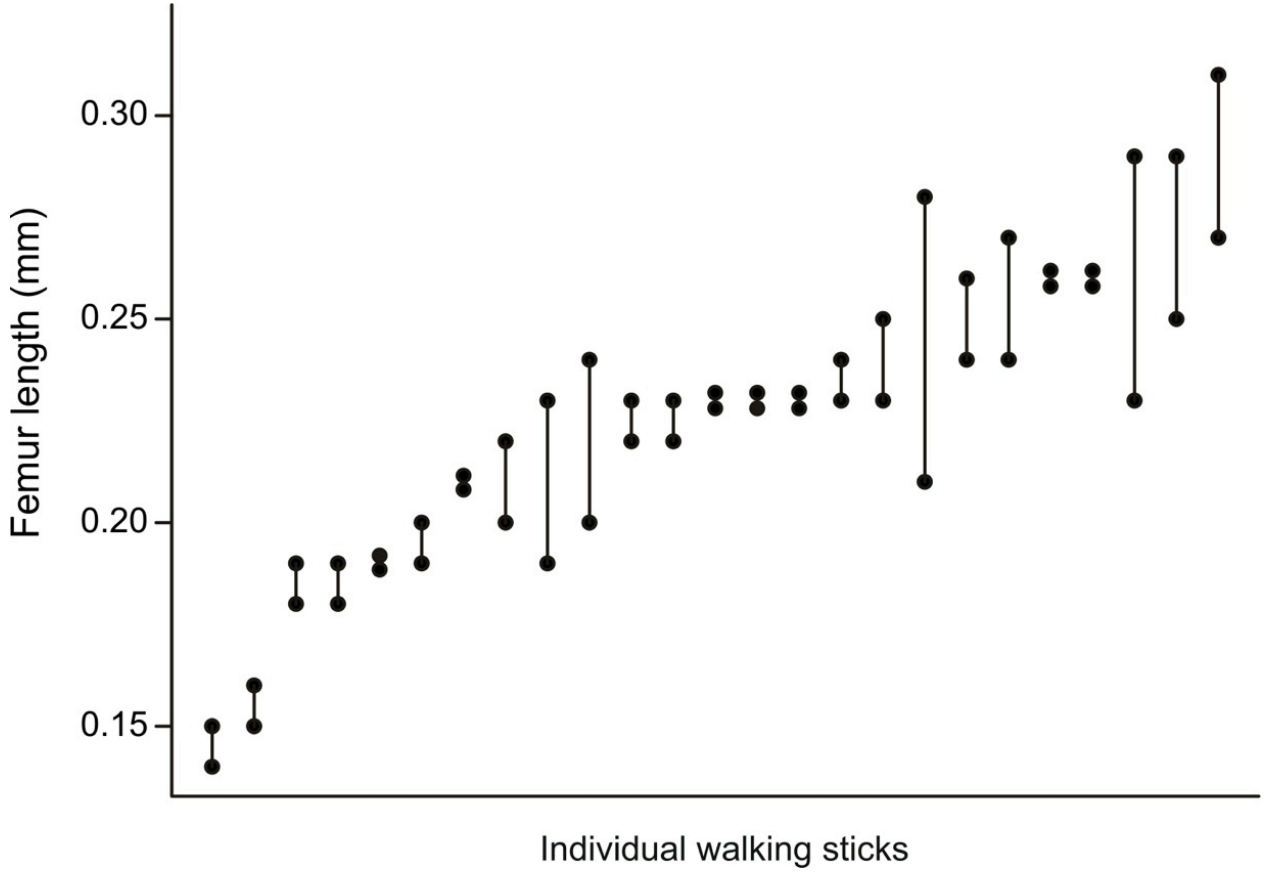
Notice that there are two formulas provided to lme:

Formula for the **fixed** effect:

```
fixed = femurlength ~ 1
```

Formula for the ***random*** effect:

```
Random = ~1|individual
```



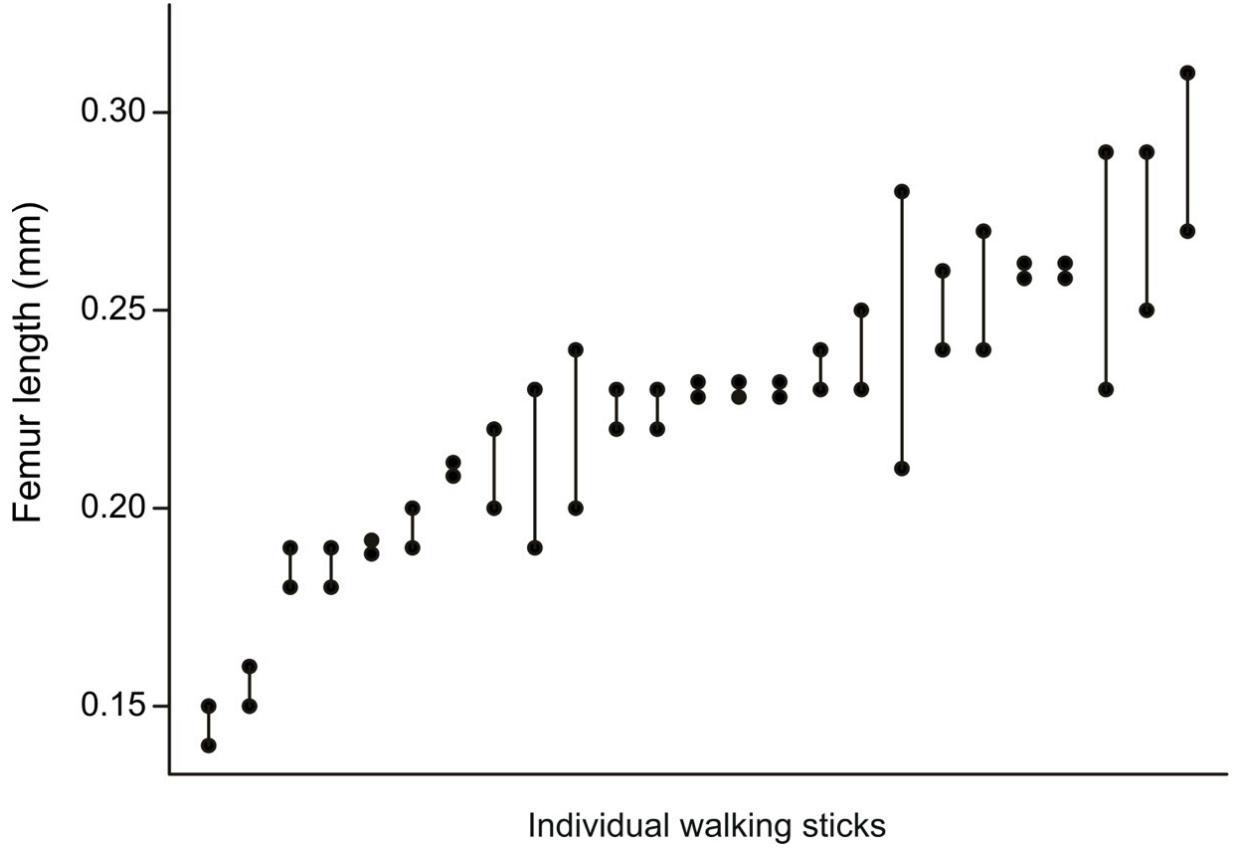
Example 1: Study of "repeatability" (simple nested design)

The ***random*** formula instructs R to fit a constant (an intercept) to the two measurements within each individual. This yields a fitted value for each individual.

```
Random = ~1|individual
```

The **fixed** formula instructs R to fit a constant (intercept) to the fitted values from each random group (each individual)

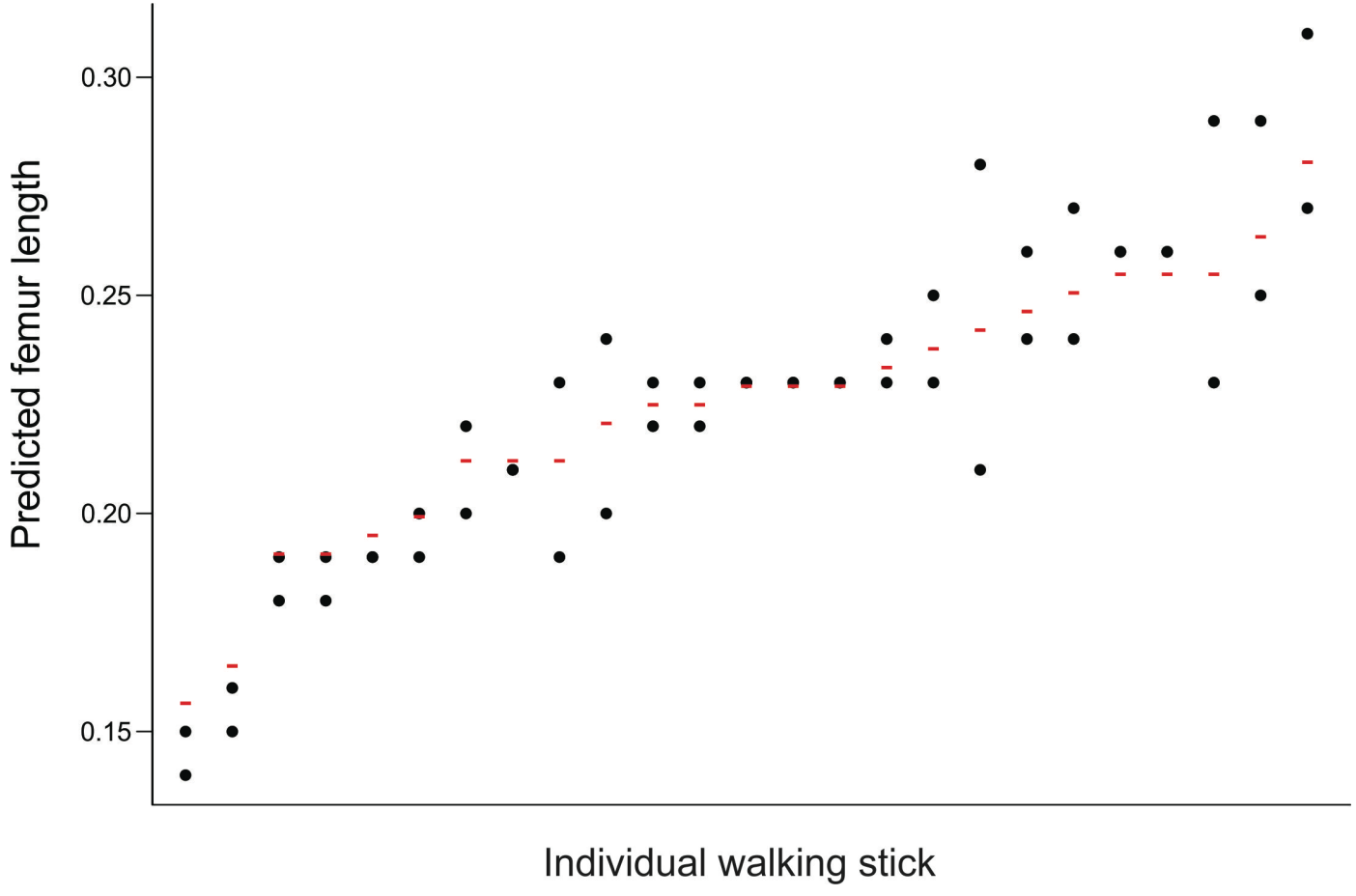
```
fixed = femurlength ~ 1
```



Example 1: Study of "repeatability" (simple nested design)

```
z <- lme(femurlength ~ 1, random = ~1|individual)
fitted(z) # yields best linear unbiased predictors (BLUPs):
```

The BLUP's are not the means for each insect. They are "shrunk" towards the centre compared with the group means.



Example 1: Study of "repeatability" (simple nested design)

`VarCorr(z)` yields the variance components

	Variance	StdDev	
(Intercept)	0.00105	0.0325	= variance among groups (individual insects)
Residual	0.00036	0.0189	= variance within groups (repeated measures)

We can use these quantities to calculate

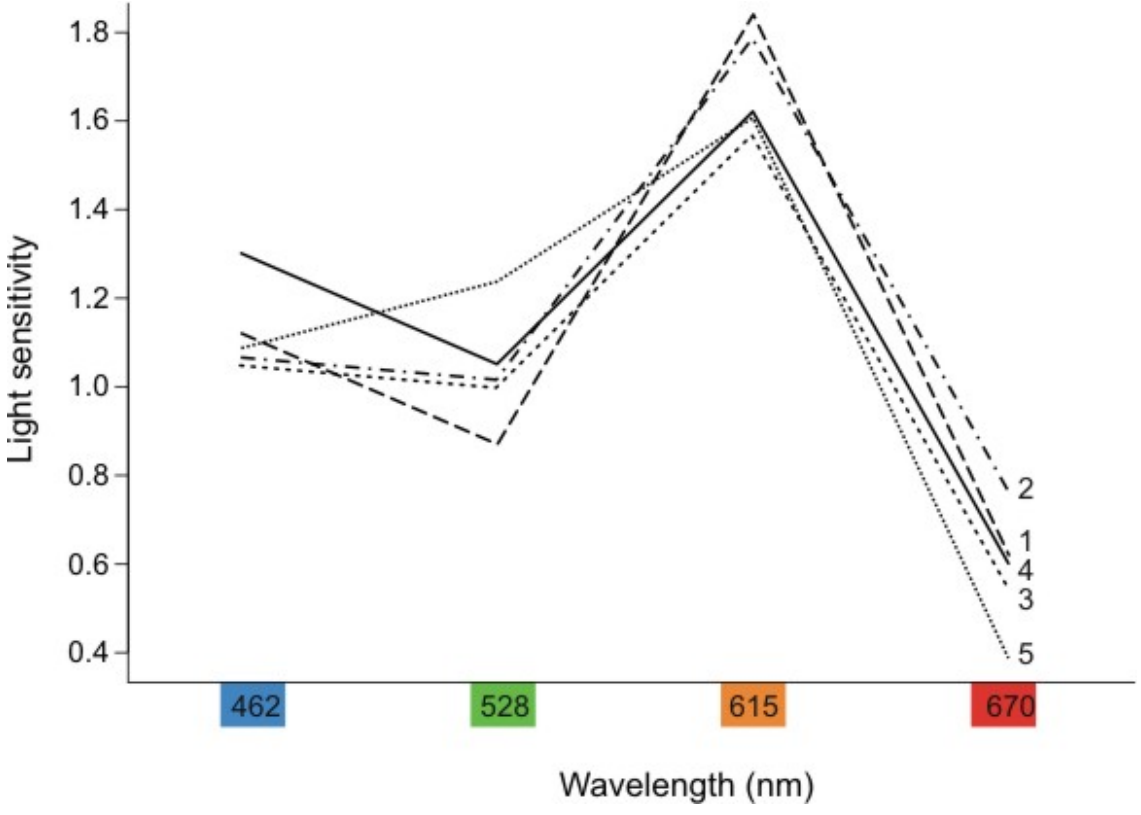
repeatability = $\sigma^2_{among} / (\sigma^2_{among} + \sigma^2_{within})$

estimate of repeatability = 0.00105 / (0.00105 + 0.00036)

= **0.75**

Example 2: “Subjects by treatment” repeated measures 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 has high sensitivity --- it can detect a low light intensity.

Factors:

Wavelength (fixed, repeated measure)

Fish (random)

Light sensitivity of 5 goldfish to specific wavelengths of light.

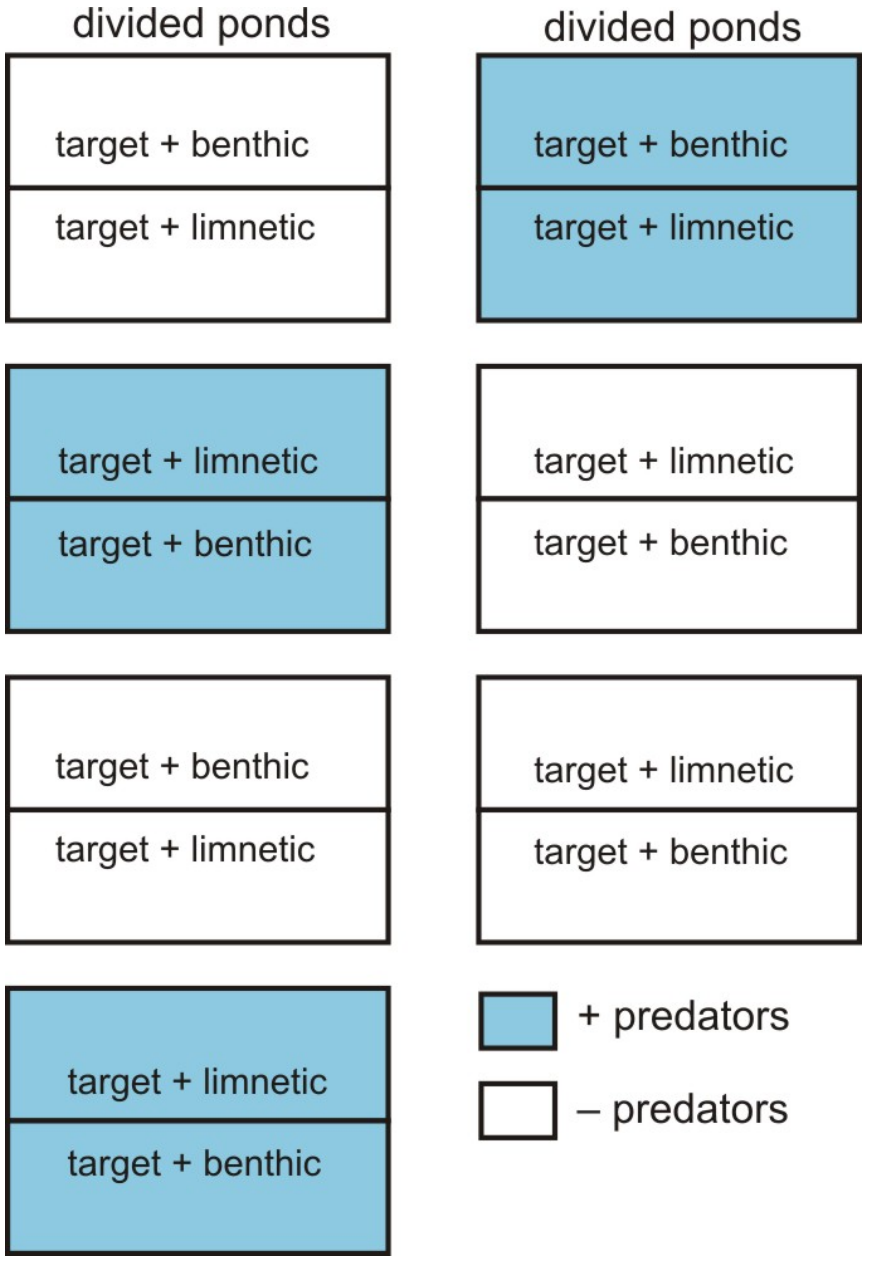
Example 3: Split plot design

Factors:

Competition treatment (fixed)

Predation treatment (fixed)

Pond (random)



Assumptions of linear mixed-effects models

- Variation within groups follows a normal distribution with equal variance among groups.
- Groups are randomly sampled from a “population” of groups (i.e., are independent and sampled without bias).
- Group effects have a normal distribution.
- Replicates within groups are also randomly sampled (i.e. independent and sampled without bias).
- No carry-over between repeated measurements on the same subject.
- Sphericity (see below).

Example 4: “Subjects by trials” repeated measures design

Ecology, 65(6), 1984, pp. 1780–1786

GRANIVORY IN A DESERT ECOSYSTEM: EXPERIMENTAL EVIDENCE FOR INDIRECT FACILITATION OF ANTS BY RODENTS[1]

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AND

R. S. INOUYE[2] AND J. H. BROWN
Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721 USA

Factors:

Rodent treatment (fixed)

Time (fixed, repeated measure)

there may be carryover effects

can't randomize time

Plot (random)

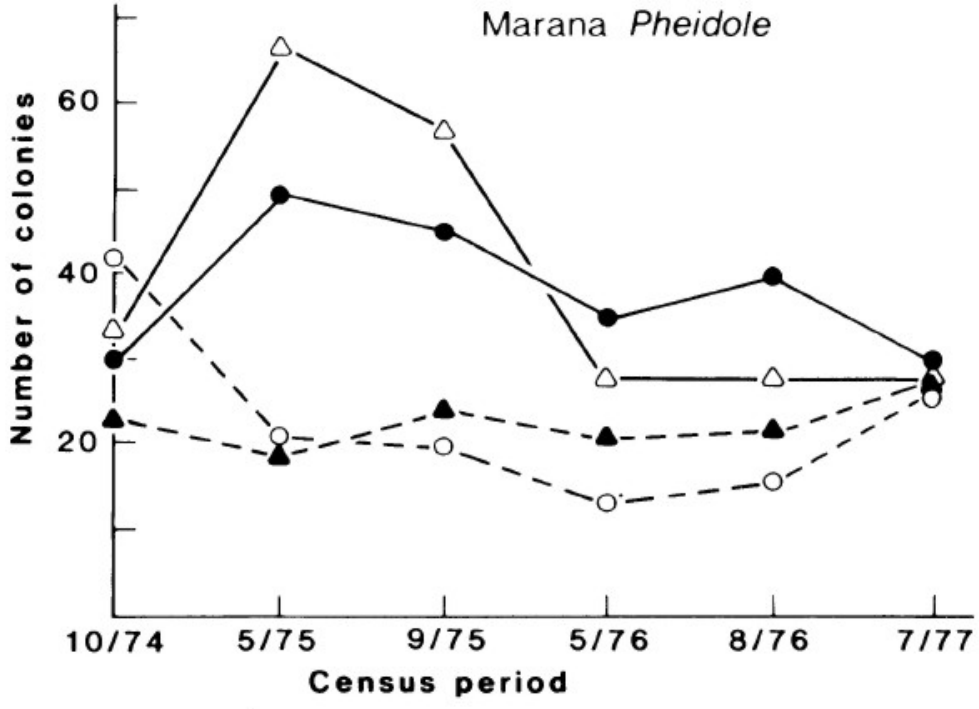


FIG. 3. Changes in density of *Pheidole* spp. (including *P. xerophila tucsonica*, *P. sitarches*, and *P. gilvescens*) on two rodent removal plots (——) and two control plots (— —) at Marana, Arizona over a 2¾-yr period.

Example 4: Warning about "subjects by trials" repeated measures design

Factors:

Rodent treatment (fixed)

Time (fixed, repeated measure)

Plot (random)

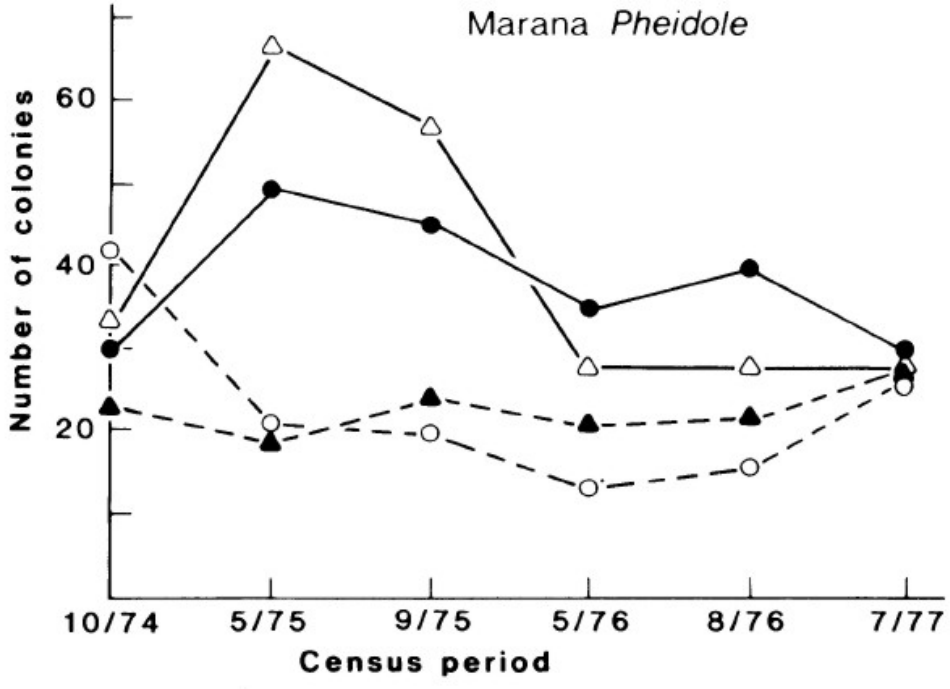
Tempting to analyze these data using a linear mixed-effects model

Fixed formula:

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

Random formula:

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



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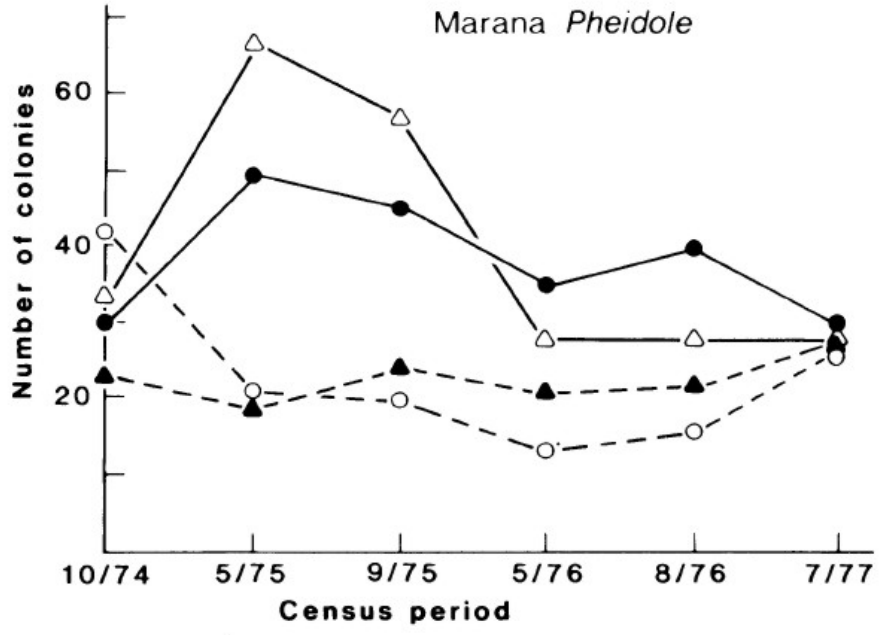
Example 4: Warning about “subjects by trials” repeated measures design

Problem: Time likely violates the sphericity assumption: the variance of the differences between values of the response variable is likely lower between nearby measurements in time than distant measurements in time.

P-values are biased downward.



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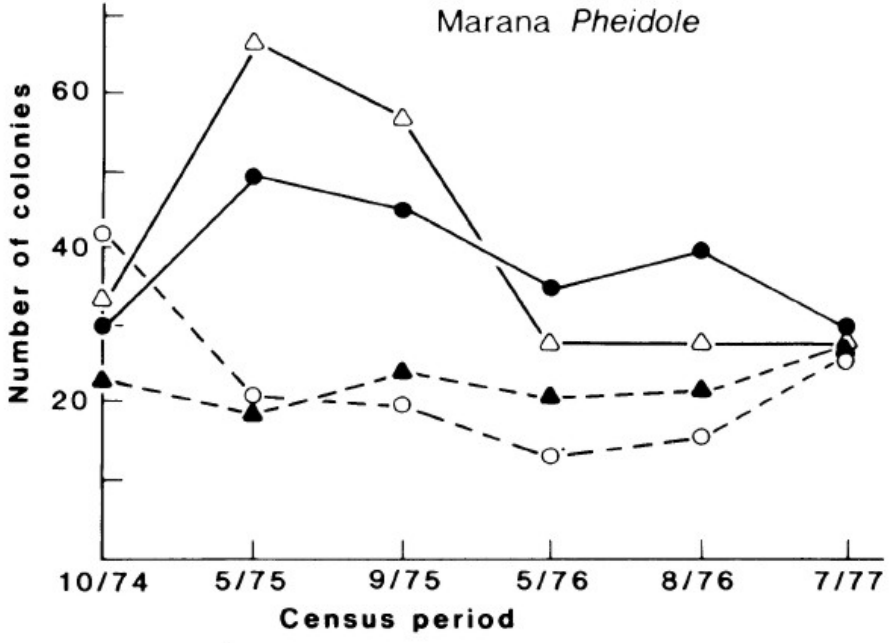
Example 4: Warning about "subjects by trials" repeated measures design

Growth curves might have the same problem.

Any repeated measures experiment in which the treatment levels are given in the same sequence (i.e., not in random order) has the same problem.

Sphericity correction is possible.

`Anova()` in `car` package: Mauchly test for sphericity, with Greenhouse-Geisser and Huynh-Feldt corrections to *P*-values.



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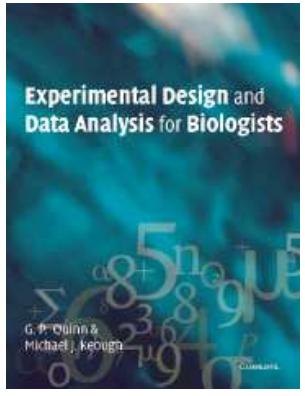
Where to get further advice

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 in R).



Quinn & Keough 2002
Experimental Design and Data Analysis for Biologists

Online books on mixed-effects models in R (see Books tab at course web site):

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

Zuur et al (2009). Mixed effects models and extensions in ecology with R.

Discussion paper for next week:

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

Presenters: Jolan & Jasmin

Moderators: Christina & Graham

Homework assignment 2

Obtain data set from supervisor/lab and fit a linear model in R.

Details on “Homework” page on course web site

Due Friday, Oct 28