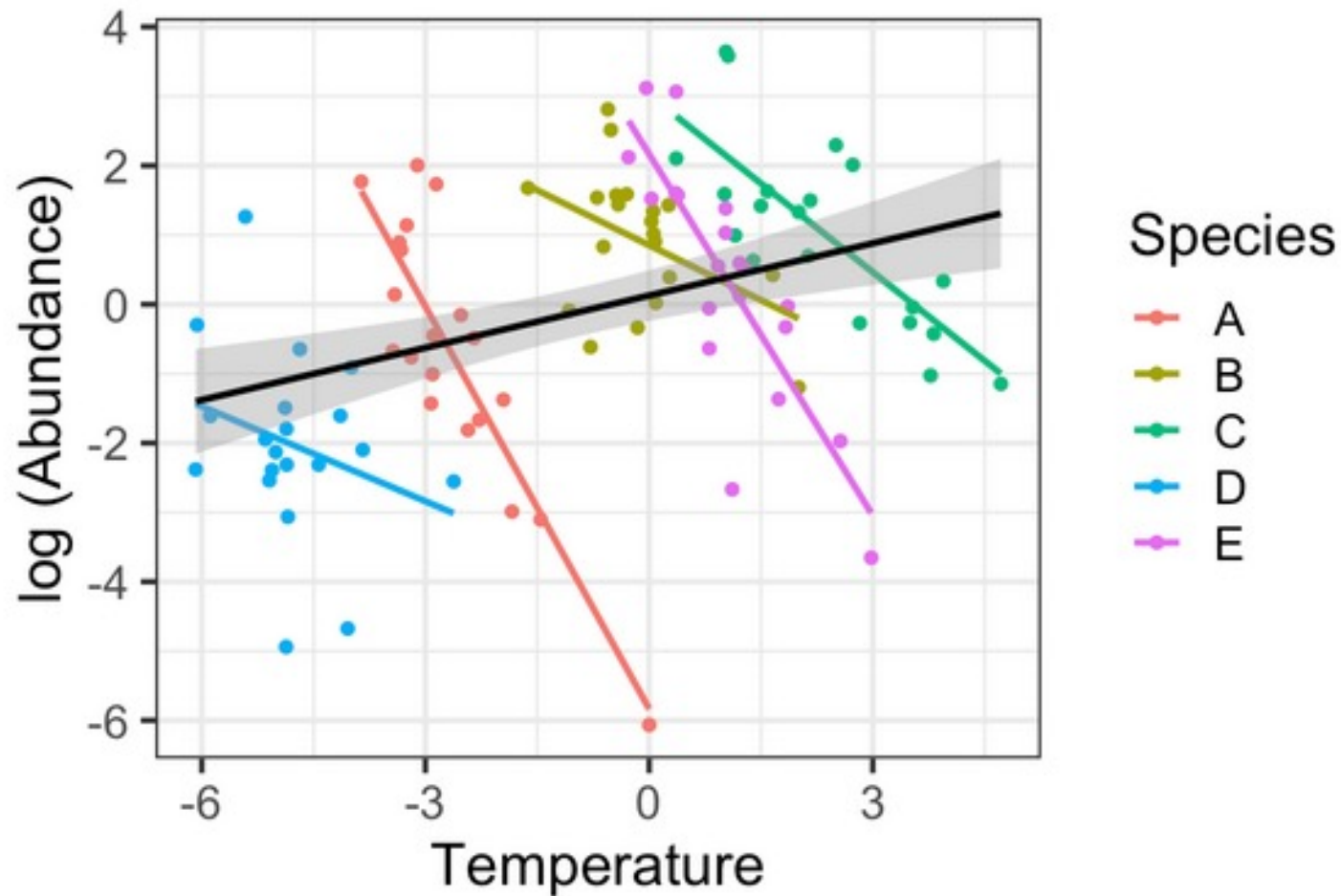


## Job trends in statistics and data analysis

With so much hinging on statistics - national and international policy, funding, corporate decision making, government commitment and research - **demand for statisticians and data analysts is expected to grow around 34% between 2014 and 2024**. This vastly outstrips the average across all jobs.

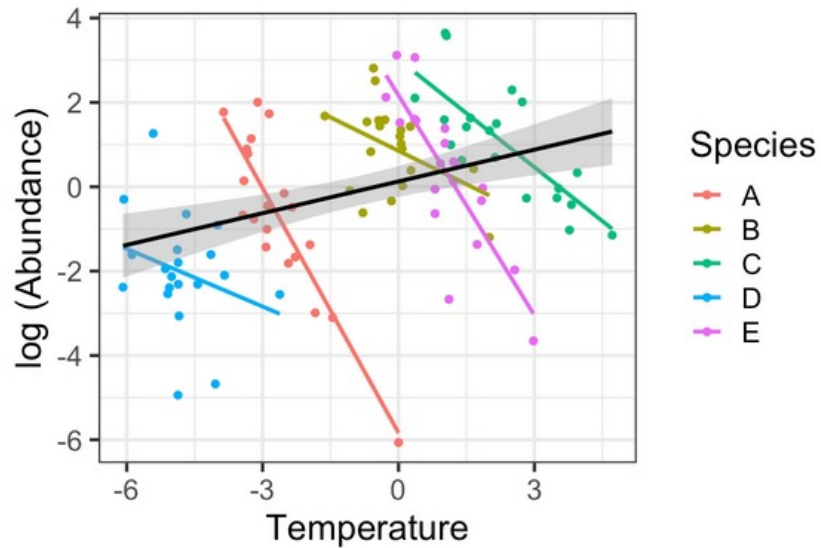
source - <https://www.environmentalscience.org/career/environmental-data-analyst>

One common feature here is that species may differ in the way they are structured by environmental and/or trait variation. This can be well described by the Simpson's paradox (Simpson 1951), which is defined "as a phenomenon in probability and statistics in which a trend appears in several groups of data but disappears or reverses when the groups are combined."



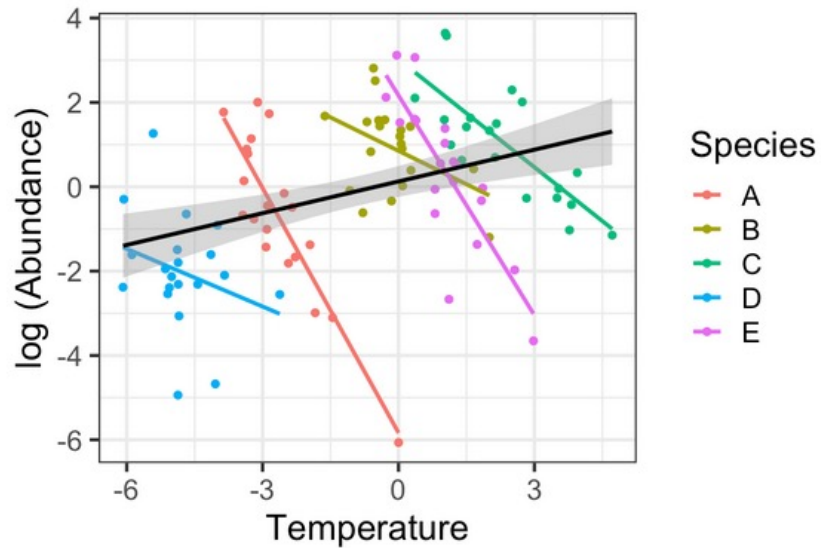
Important enough to have its own Wikipedia page:  
[https://en.wikipedia.org/wiki/Simpson%27s\\_paradox](https://en.wikipedia.org/wiki/Simpson%27s_paradox)

## Fixed effect model



```
## MODEL INFO:
## Observations: 100
## Dependent Variable: abundance
## Type: OLS linear regression
##
## MODEL FIT:
## F(1,98) = 15.13, p = 0.00
## R2 = 0.13
## Adj. R2 = 0.12
##
## Standard errors: OLS
## -----
##                               Est.   S.E.   t val.   p
## -----
## (Intercept)                 -0.08   0.18    -0.48   0.63
## scale(temperature)           0.69   0.18     3.89   0.00
## -----
##
## Continuous predictors are mean-centered and scaled by 1 s.d.
```

# Mixed effect model



```
lm.mod.intercept <- lmer(abundance ~ temperature + (1|species),data=data.Simpson)
summ(lm.mod.intercept,scale = TRUE)
```

```
## MODEL INFO:
## Observations: 100
## Dependent Variable: abundance
## Type: Mixed effects linear regression
##
## MODEL FIT:
## AIC = 343.74, BIC = 354.16
## Pseudo-R2 (fixed effects) = 0.30
## Pseudo-R2 (total) = 0.95
##
## FIXED EFFECTS:
```

	Est.	S.E.	t val.	d.f.	p
(Intercept)	-0.08	1.88	-0.04	3.77	0.97
temperature	-2.84	0.34	-8.22	97.68	0.00

## General linear models (not Generalized linear model)

	Linear Model	Common name
✓	$Y = \mu + X$	Simple linear regression
✓	$Y = \mu + A_1$	One-factorial (one-way) ANOVA
✓	$Y = \mu + A_1 + A_2 + A_1 \times A_2$	Two-factorial (two-way) ANOVA
✓	$Y = \mu + A_1 + X (+A_1 \times X)$	Analysis of Covariance (ANCOVA)
✓	$Y = \mu + X_1 + X_2 + X_3$	Multiple regression
⇒	$Y = \mu + A_1 + g + A_1 \times g$	Mixed model ANOVA
	$Y_1 + Y_2 = \mu + A_1 + A_2 + A_1 \times A_2$	Multivariate ANOVA (MANOVA)

Y (response) is a continuous variable

X (predictor) is a continuous variable

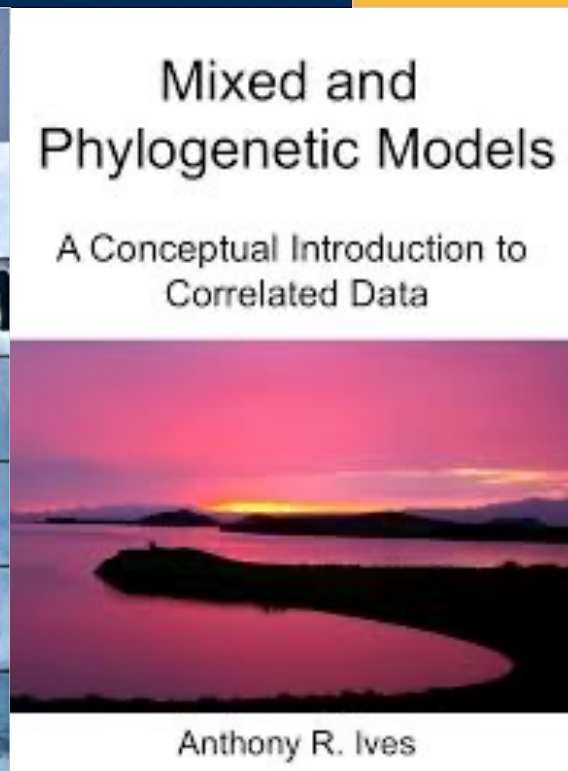
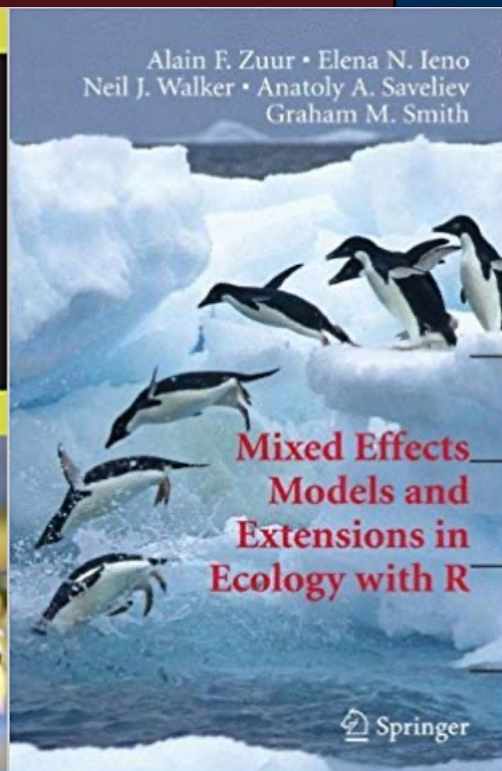
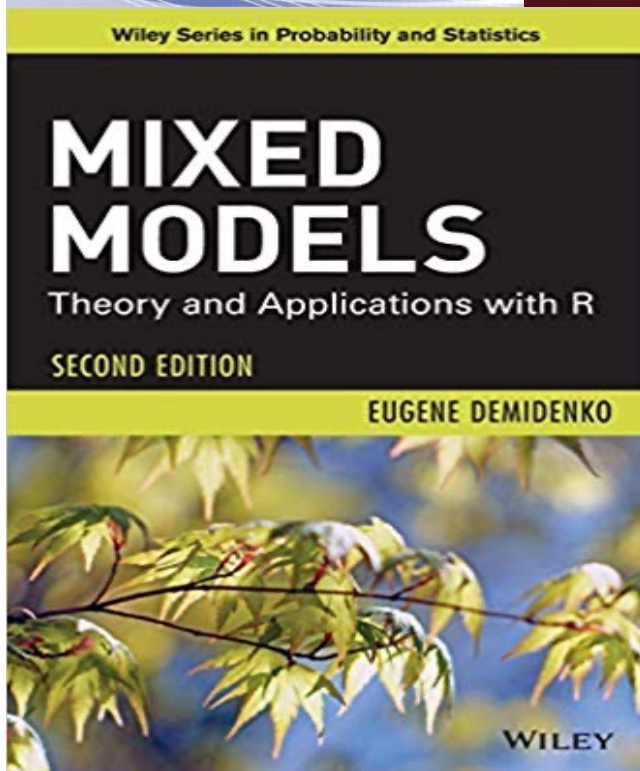
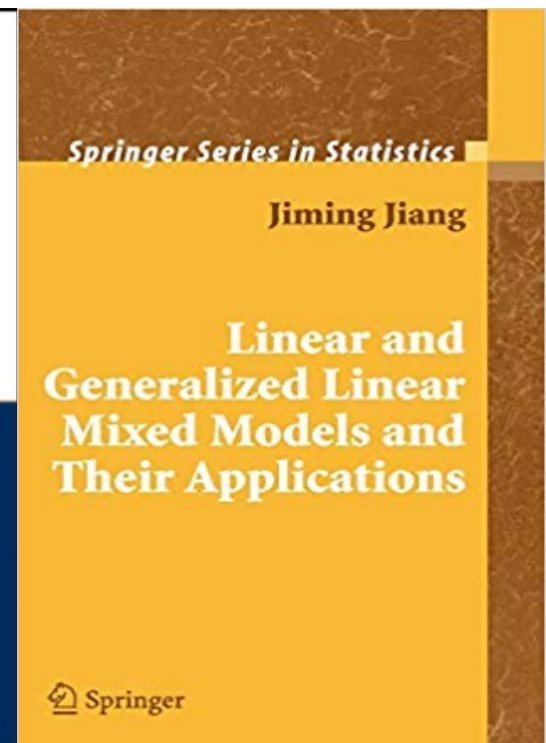
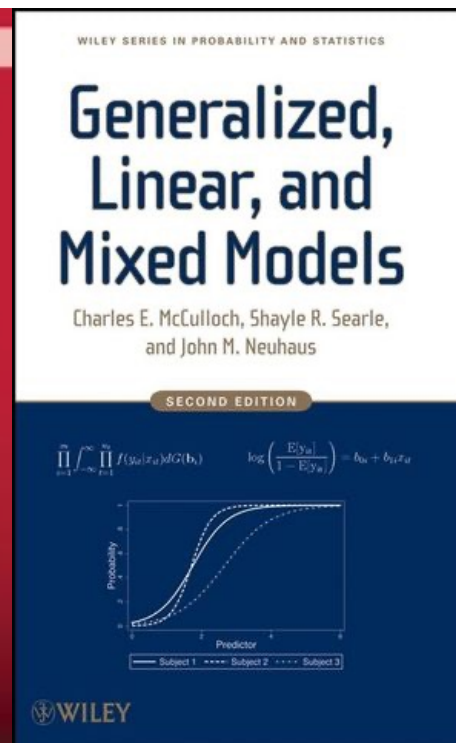
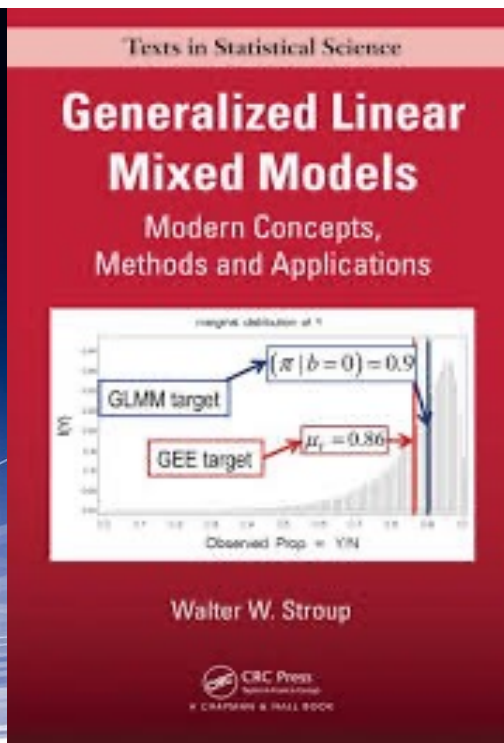
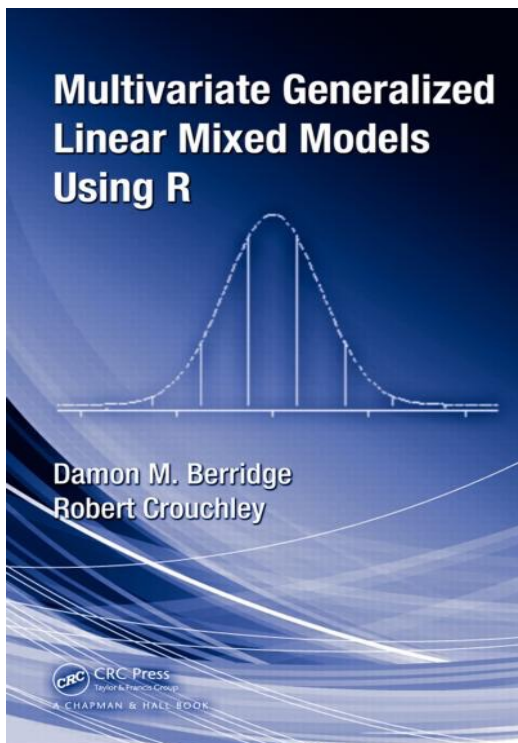
A represents categorical predictors (factors)

g represents groups of data (more on this later)

(+A<sub>1</sub> × X) - step 1 on an ANCOVA, but not in the final analysis

Multiple factors A<sub>1</sub> + A<sub>2</sub> + etc (and their interactions)



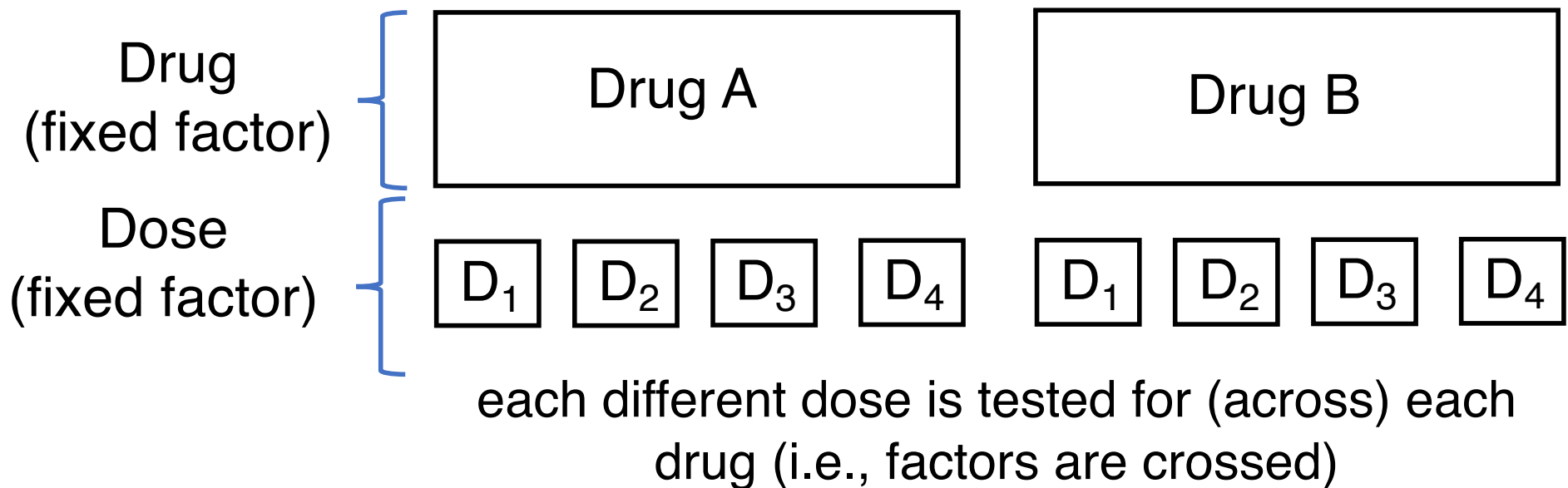


## Fixed effects are often crossed in relation to other fixed effects (e.g., typical two-way ANOVA)

**Fixed effect factor:** Data have been gathered from all the levels of the factor that are of interest.

Example: Contrasting the effects of three specific dosages of a drug on the response. "Dosage" is the factor; the three specific dosages in the experiment are the levels; there is no intent to say anything about other dosages.

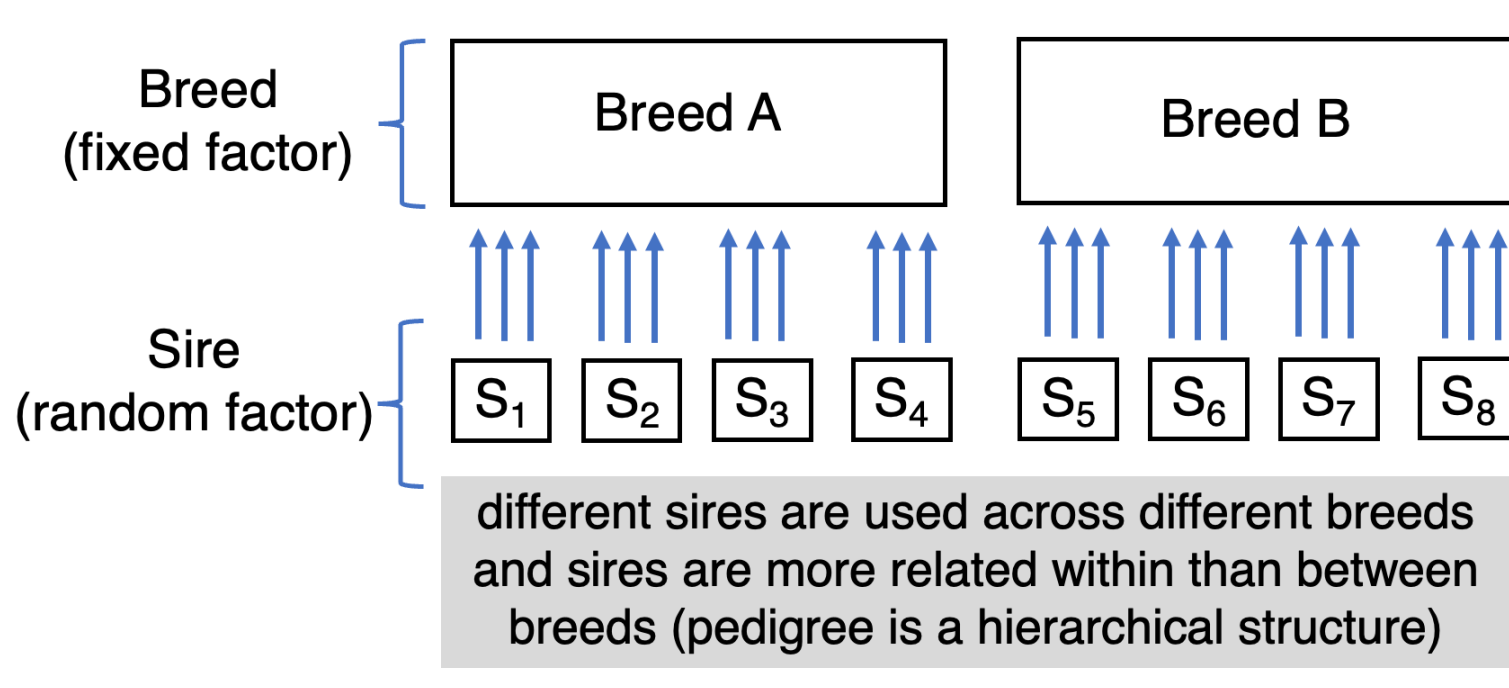
(source: <https://www.ma.utexas.edu/users/mks/statmistakes/fixedvsrandom.html>)



## Random versus fixed effects – a hierarchical view

**Random effect factor (sometime referred as a variance component model):** The factor has many possible levels. Although there is interest in all possible levels, only a random sample of levels can be included in the data (either due to lack of knowledge all all possible levels, or costs, or both, or other issues).

*Example:* In an animal breeding experiment conducted to estimate the breeding value of sires (male parents) from a certain breed, several sires were **randomly selected from a population** and each sire was mated with several dams (mother). The weights of all the newborn animals were recorded.

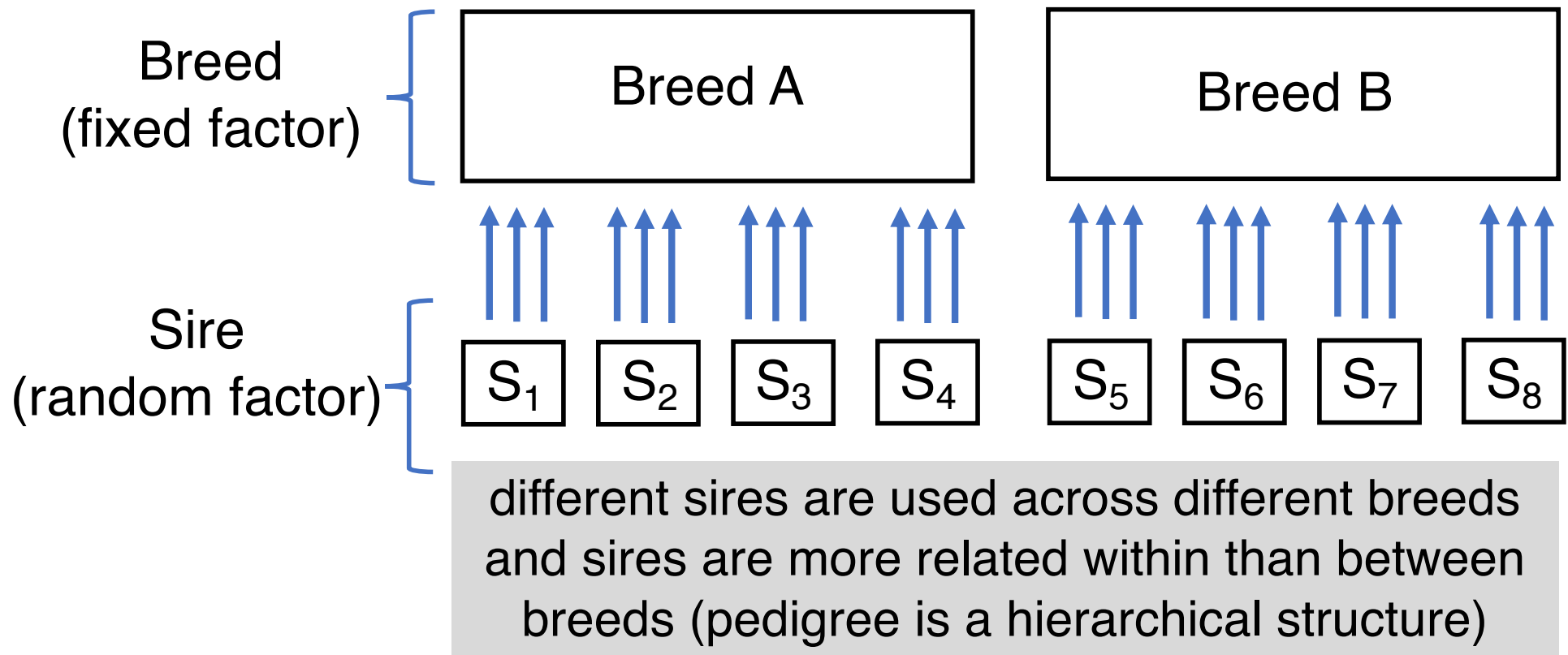




## Random *versus* fixed effects – a hierarchical view

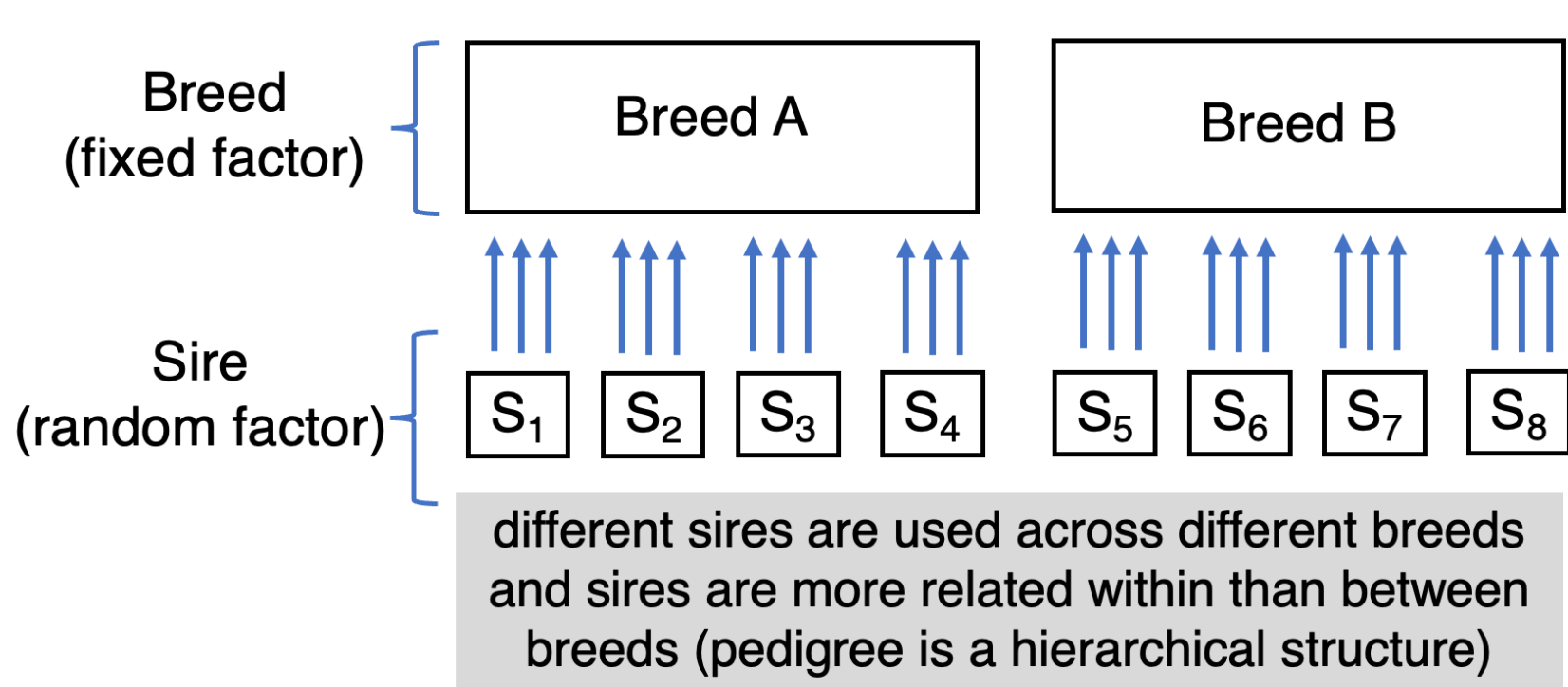
The factor is “sire” (male gamete). The analysis will not estimate the effect of each of the sires in the sample; instead, it will **estimate the variability attributable to the factor “sire” (male parents)**.

It is a type of hierarchical linear model, which assumes that the data being analysed are drawn from a hierarchy of different populations whose differences relate to that hierarchy.



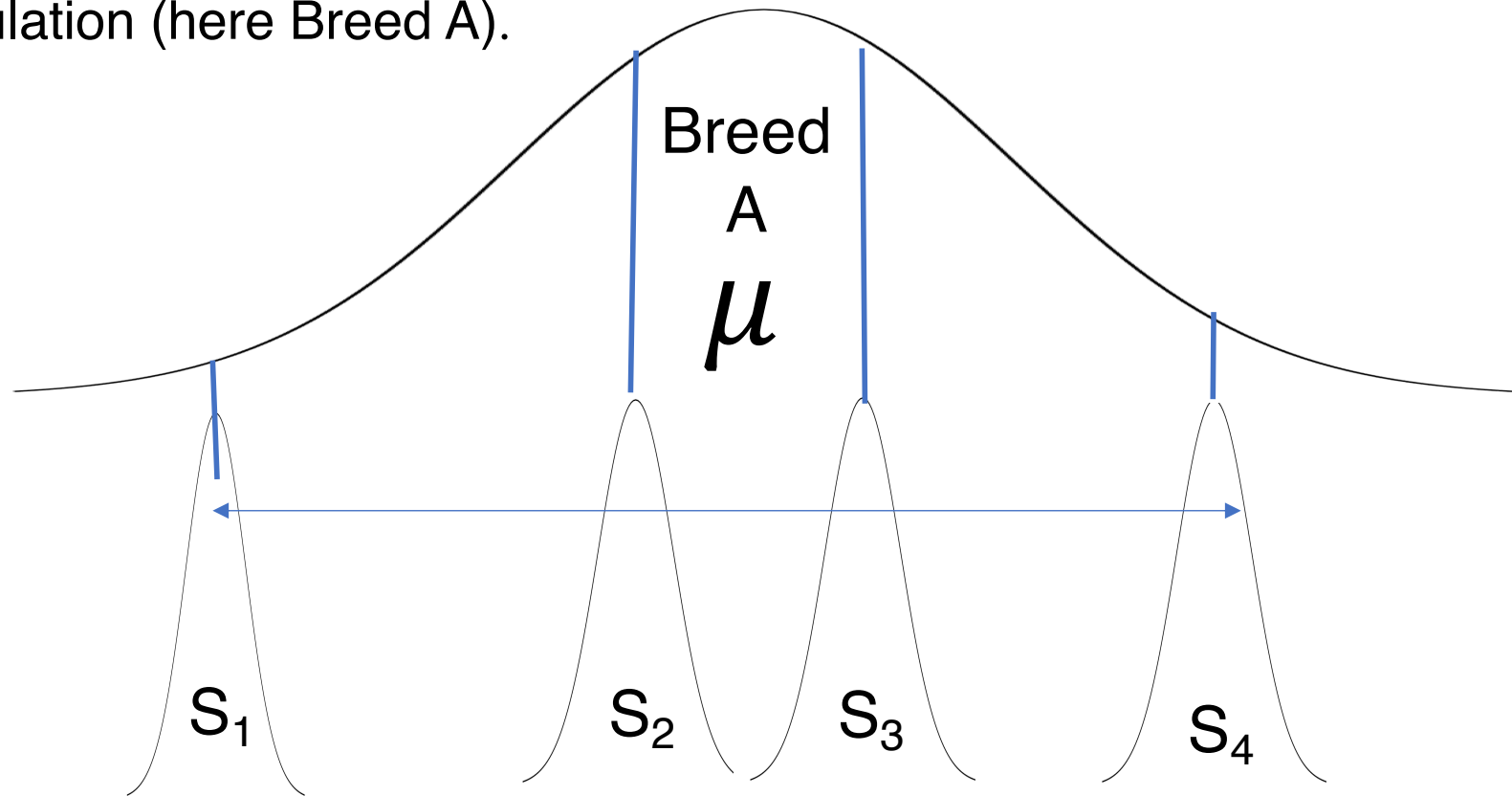
Random effects are often ***hierarchical*** in relation to fixed effects

Here, sires are a random variable (factor) that will change from study to study. In contrast (based on another example), drug levels are of true interest that won't change from study to study.



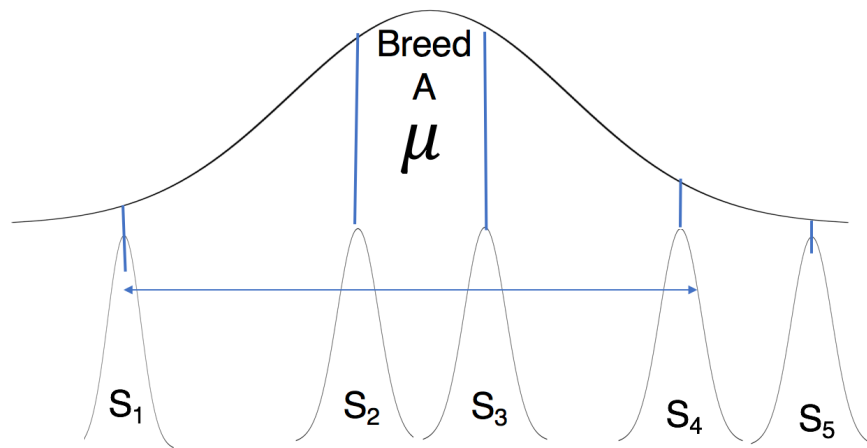
## Random effects – let's focus on a single breed

In a one-way (factor) random effect ANOVA, the goal is to estimate the variance of a breed (variation among sires). The sires are merely a sample from which inferences are to be made concerning the single population (here Breed A).



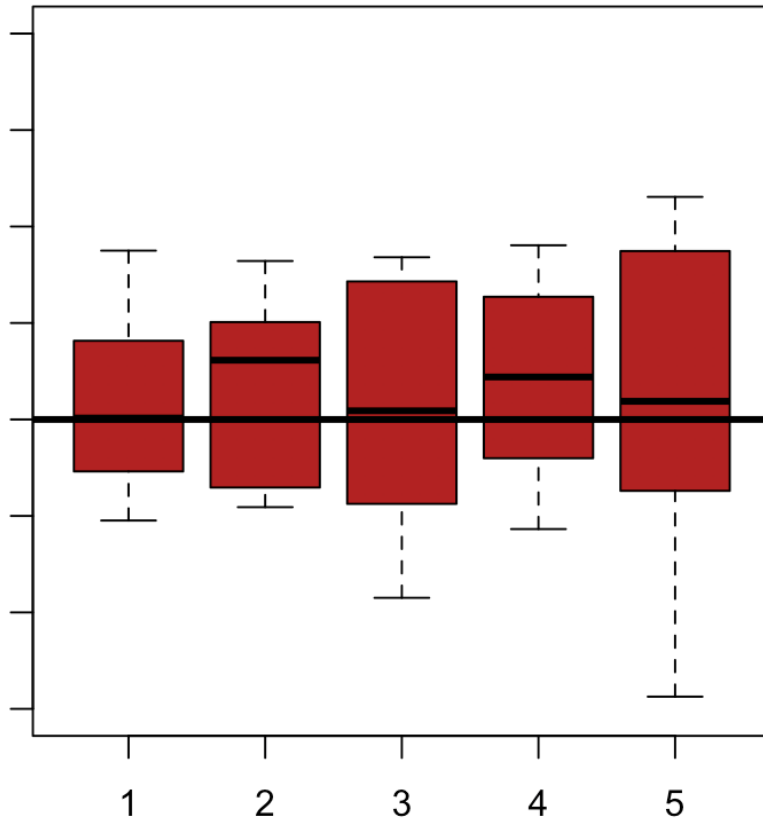
weights of all the newborn animals (1 per dam – female parent) were recorded for each sire

**Do sirens vary in their newborn weights? This can be answered by assessing whether there is more variation between sirens than within sirens.**

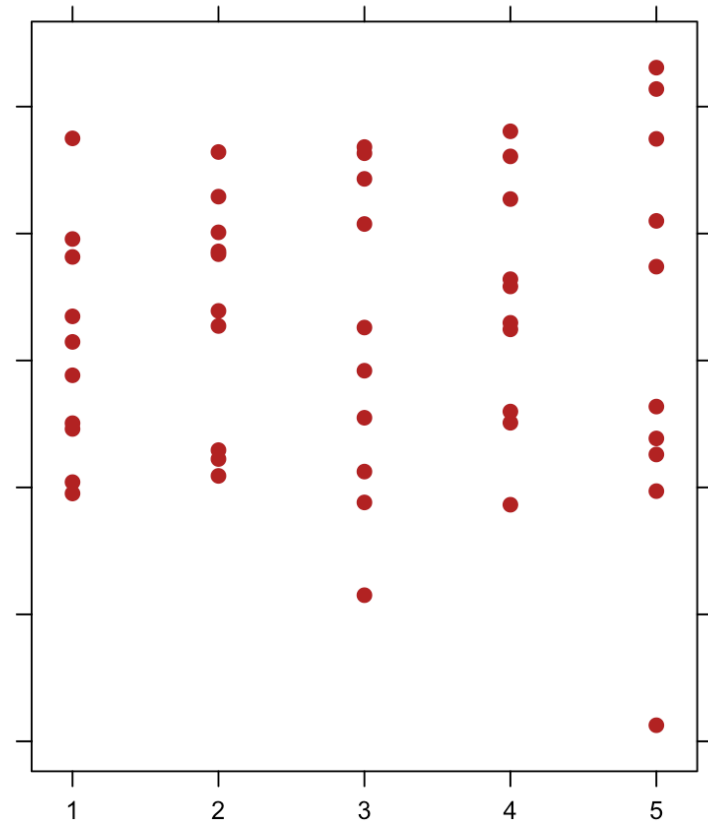


variance within groups and  
variance within level  
**(LOW VARIATION)**

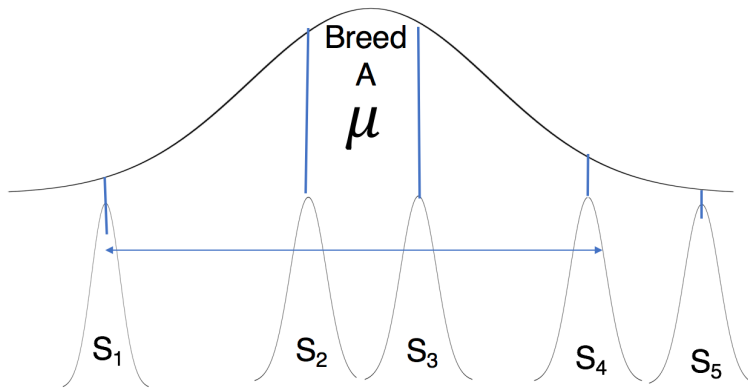
Weight (newborns)



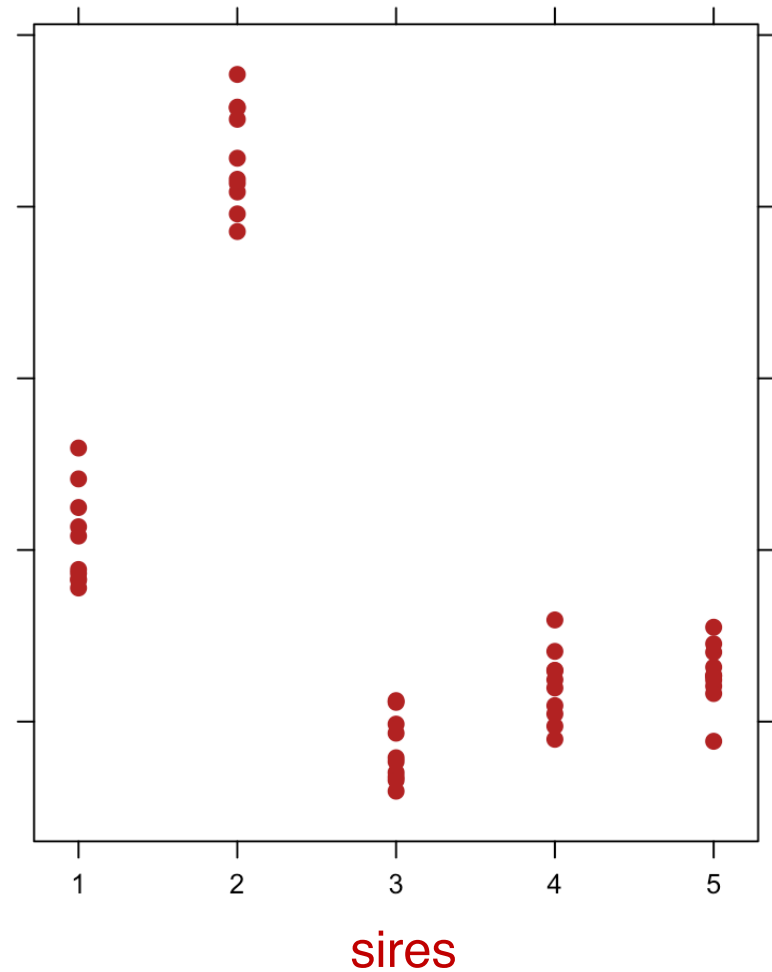
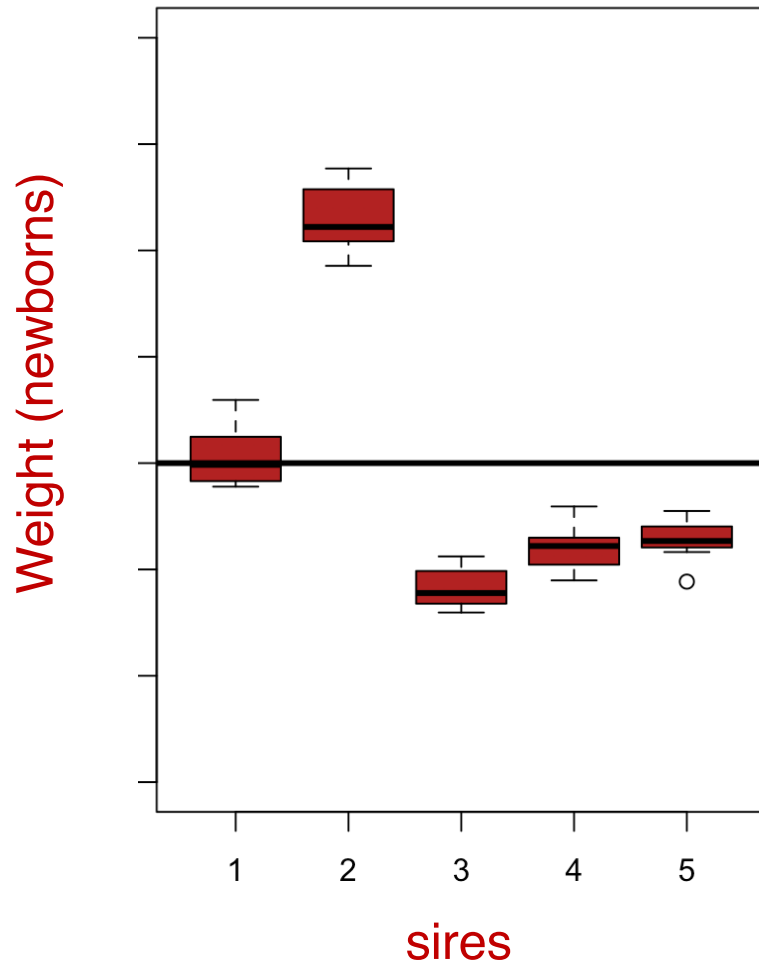
sires



sires



variance within groups and  
variance within level  
**(HIGH VARIATION)**



## Fixed versus random effects may depend on the question and not always the data

STUDY: Suppose five cuts of meat are taken from each of three pigs, all from the same breed, and the fat content is measured in each cut.

**FIXED EFFECT QUESTION** – Do the different cuts differ in their fat content? One-way (fixed) ANOVA with five treatment levels (cuts) and three replicates (observations) per cut (pigs).





```
pig1 <- c(1.1,2.1,3.1,4.1,5.1)
pig2 <- c(1.2,2.2,3.2,4.2,5.1)
pig3 <- c(0.9,2.3,3.1,4.2,5.2)

cut.weights <- c(pig1,pig2,pig3)
cut.factor <- as.factor(rep(1:5,3))
pig <- as.factor(c(rep(1,5),rep(2,5),rep(3,5)))
View(cbind(cut.weights,cut.factor,pig))
anova(lm(cut.weights~cut.factor))
```

	cut.weights	cut.factor	pig
1	1.1	1	1
2	2.1	2	1
3	3.1	3	1
4	4.1	4	1
5	5.1	5	1
6	1.2	1	2
7	2.2	2	2
8	3.2	3	2
9	4.2	4	2
10	5.1	5	2
11	0.9	1	3
12	2.3	2	3
13	3.1	3	3
14	4.2	4	3
15	5.2	5	3



```
pig1 <- c(1.1,2.1,3.1,4.1,5.1)
pig2 <- c(1.2,2.2,3.2,4.2,5.1)
pig3 <- c(0.9,2.3,3.1,4.2,5.2)

cut.weights <- c(pig1,pig2,pig3)
cut.factor <- as.factor(rep(1:5,3))
pig <- as.factor(c(rep(1,5),rep(2,5),rep(3,5)))
View(cbind(cut.weights,cut.factor,pig))
anova(lm(cut.weights~cut.factor))
```



```
> anova(lm(cut.weights~cut.factor))
Analysis of Variance Table
```

Response: cut.weights

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
cut.factor	4	30.6293	7.6573	883.54	1.07e-12 ***
Residuals	10	0.0867	0.0087		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

	cut.weights	cut.factor	pig
1	1.1	1	1
2	2.1	2	1
3	3.1	3	1
4	4.1	4	1
5	5.1	5	1
6	1.2	1	2
7	2.2	2	2
8	3.2	3	2
9	4.2	4	2
10	5.1	5	2
11	0.9	1	3
12	2.3	2	3
13	3.1	3	3
14	4.2	4	3
15	5.2	5	3

## Fixed versus random effects may depend on the question and not always the data

STUDY: Suppose five cuts of meat are taken from each of three pigs, all from the same breed, and the fat content is measured in each cut.

FIXED EFFECT QUESTION – Do the different cuts differ in their fat content? One-way (fixed) ANOVA with five treatment levels (cuts) and three replicates per cut (pigs).

**RANDOM EFFECT QUESTION** - Is there more variation in fat content among or within pigs (i.e., animal-to-animal and within-animal variation)? A fat pig could have their cuts fatter (i.e., hierarchical variation).

In this case, the three pigs selected are not of interest. This would be a one-way random effects ANOVA.

Note that there is more variation within pigs  
(i.e., among cuts within pigs) than between pigs

```

pig1 <- c(1.1,2.1,3.1,4.1,5.1)
pig2 <- c(1.2,2.2,3.2,4.2,5.1)
pig3 <- c(0.9,2.3,3.1,4.2,5.2)

cut.weights <- c(pig1,pig2,pig3)
cut.factor <- as.factor(rep(1:5,3))
pig <- as.factor(c(rep(1,5),rep(2,5),rep(3,5)))
View(cbind(cut.weights,cut.factor,pig))
anova(lm(cut.weights~cut.factor))

```

```

library(nlme)
gls(cut.weights ~ 1, correlation=corCompSymm(form= ~ 1 | pig))

```

Rho refers to intraclass  
(here pig) correlation

A negative or low ICC suggests that  
there is more variability within  
subjects (here pigs) than between  
subjects.

```

Correlation Structure: Compound symmetry
Formula: ~1 | pig
Parameter estimate(s):
      Rho
-0.2490236
Degrees of freedom: 15 total; 14 residual
Residual standard error: 1.431177

```

Now, these data have more variation between pigs  
(i.e., among cuts within pigs) than within pigs

```
pig1 <- c(1.1,2.1,3.1,4.1,5.1)
pig2 <- c(1.2,2.2,3.2,4.2,5.1) + 10
pig3 <- c(0.9,2.3,3.1,4.2,5.2) + 100
cut.weights <- c(pig1,pig2,pig3)
gls(cut.weights ~ 1, correlation=corCompSymm(form= ~ 1 | pig))
```

```
Correlation Structure: Compound symmetry
Formula: ~1 | pig
Parameter estimate(s):
      Rho
0.9991573
Degrees of freedom: 15 total; 14 residual
Residual standard error: 55.09793
```

Rho refers to intraclass  
(here pig) correlation

A high positive ICC suggests that there  
is less variability within subjects (here  
pigs) than between subjects.

	cut.weights	cut.factor	pig
1	1.1	1	1
2	2.1	2	1
3	3.1	3	1
4	4.1	4	1
5	5.1	5	1
6	1.2	1	2
7	2.2	2	2
8	3.2	3	2
9	4.2	4	2
10	5.1	5	2
11	0.9	1	3
12	2.3	2	3
13	3.1	3	3
14	4.2	4	3
15	5.2	5	3

## More on the hierarchical nature of data

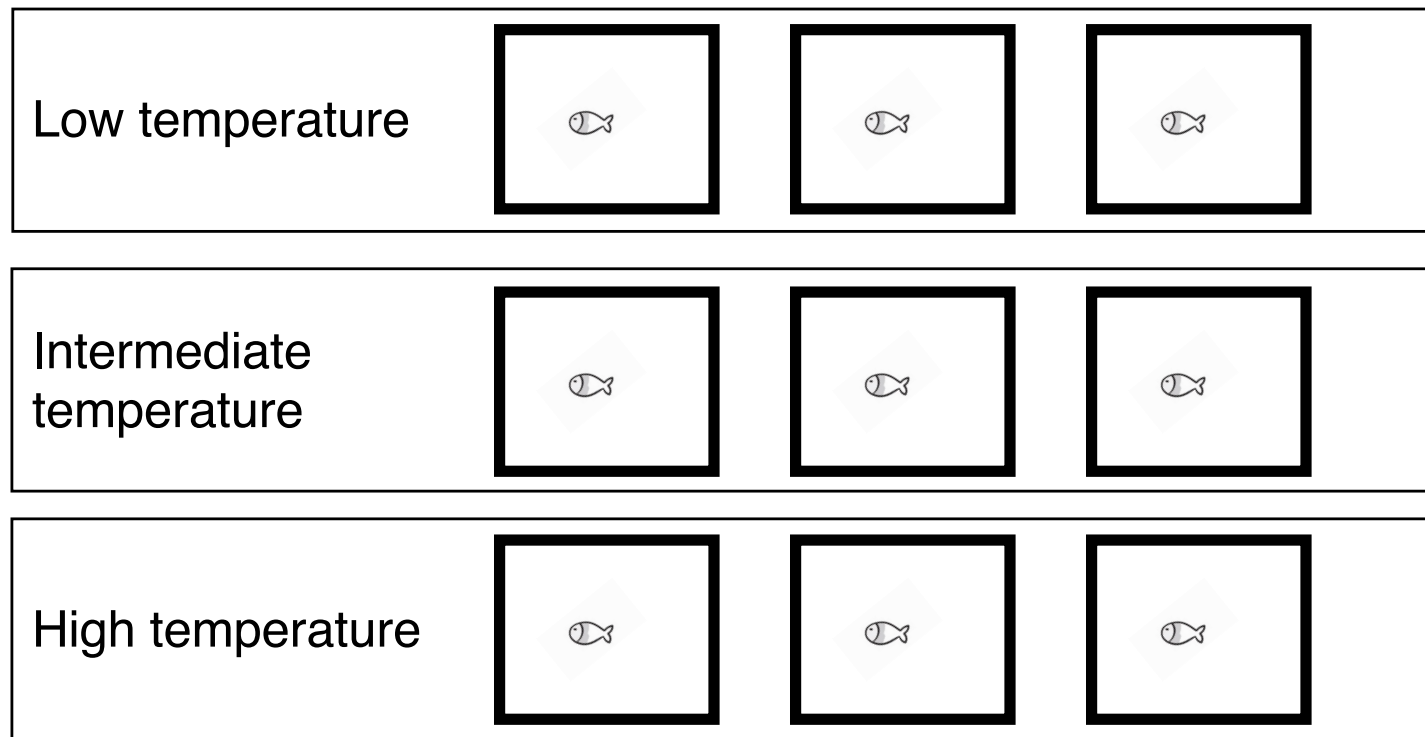




The advantages of mixed models - increase statistical power and estimation accuracy through dependent replication and design convenience (particularly in observational studies).

**Do we need a random effect here?**

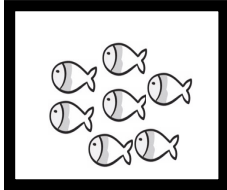
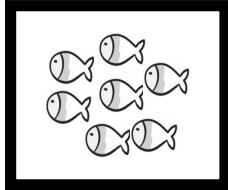
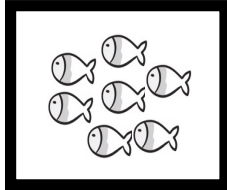
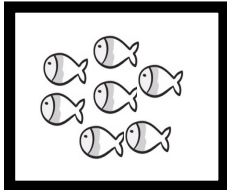
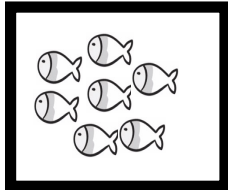
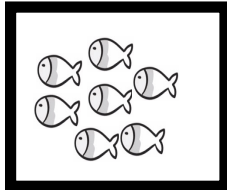
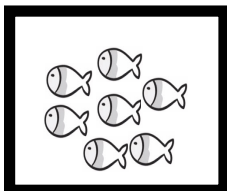
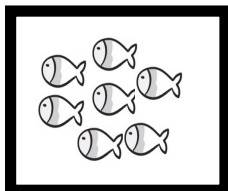
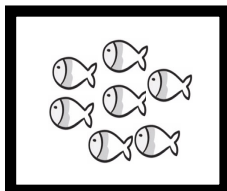
Effects of temperature on fish growth  
(difference in growth begin/end of study)



The advantages of mixed models - increase statistical power and estimation accuracy through dependent replication and design convenience (particularly in observational studies).

**Do we need a random effect here?**

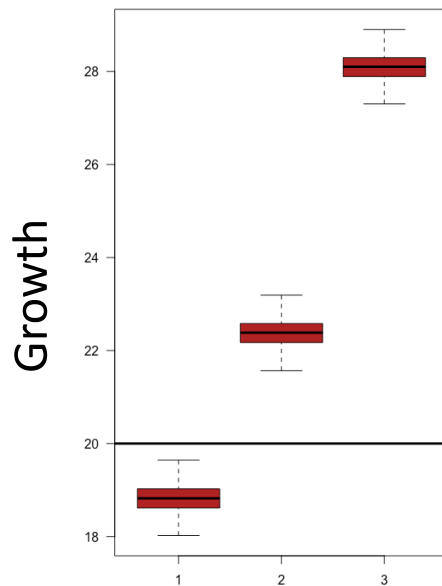
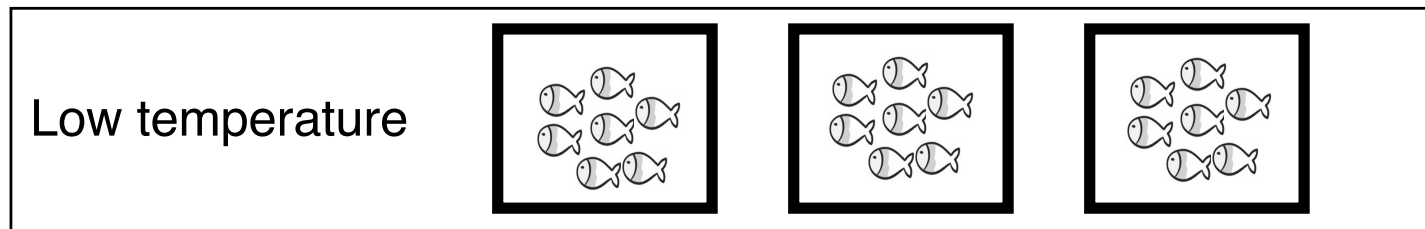
Effects of temperature on fish growth  
(difference in growth begin/end of study)

Low temperature			
Intermediate temperature			
High temperature			

The advantages of mixed models - increase statistical power and estimation accuracy through dependent replication and design convenience (particularly in observational studies).

### Do we need a random effect here?

Effects of temperature on fish growth  
(difference in growth begin/end of study)



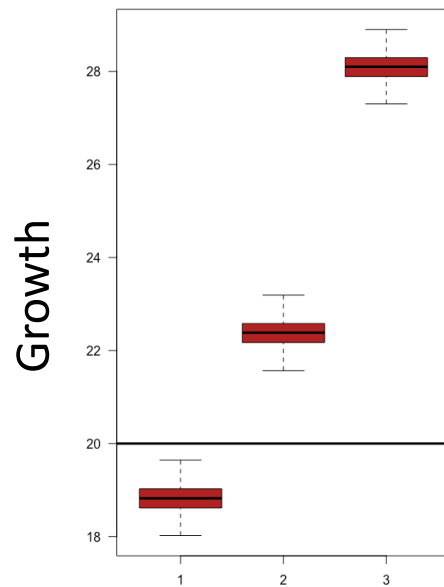
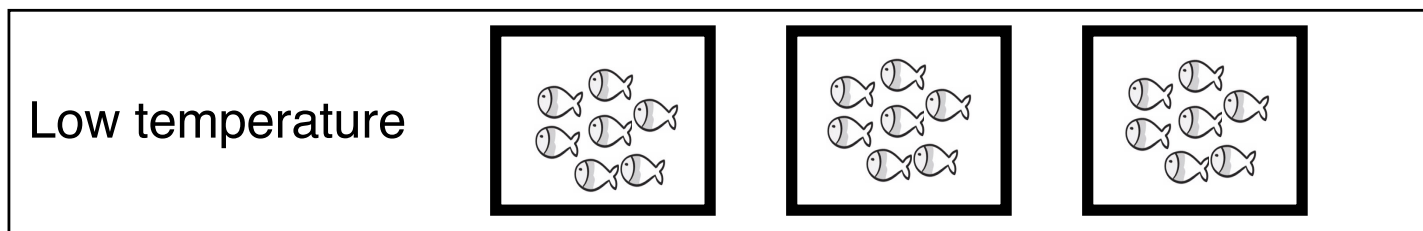
A positive or low ICC suggests that there is less variability within subjects (here aquaria) than between subjects.

Tank low rho (ICC)

The advantages of mixed models - increase statistical power and estimation accuracy through dependent replication and design convenience (particularly in observational studies).

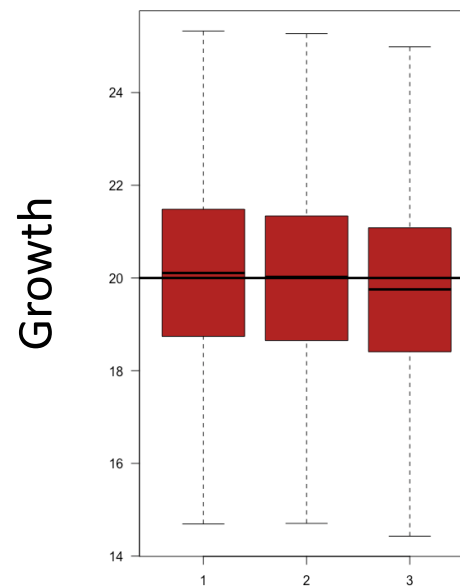
### Do we need a random effect here?

Effects of temperature on fish growth  
(difference in growth begin/end of study)



Tank low rho (ICC)

versus



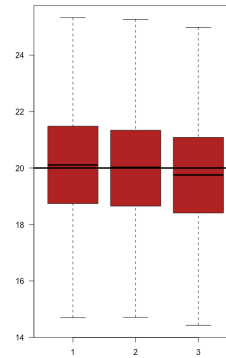
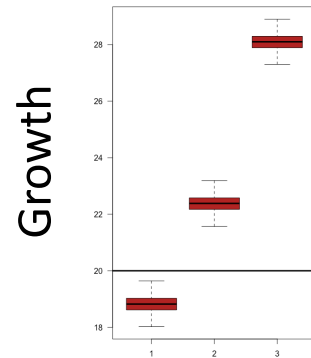
Tank

high rho (ICC)

A high ICC suggests that there is more variability within subjects (here aquaria) than between subjects.

Do we need a random effect here? Which experimental results should we trust the most?  
(note that scales of the response are about the same)

Low temperature

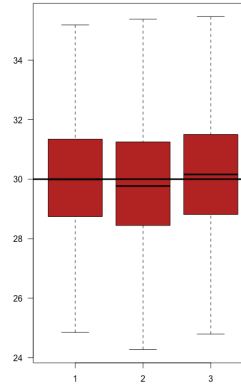
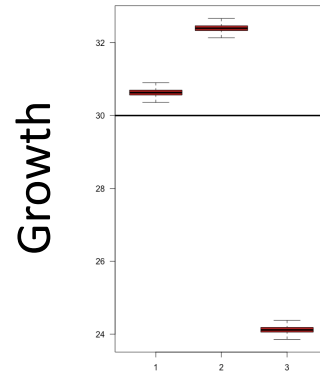


Average  
within  
treatment =  
20g

Fixed factor =  
temperature

Random factor =  
aquarium

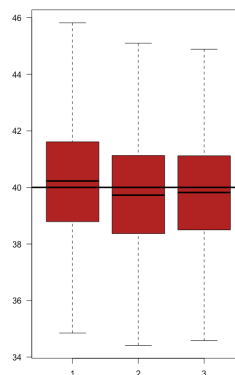
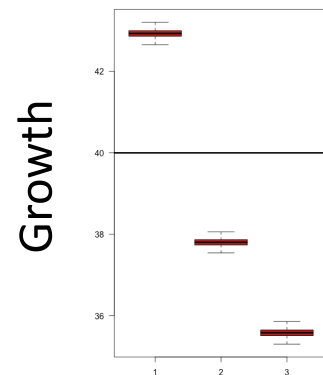
Intermediate  
temperature



Average  
within  
treatment =  
30g

Compare the two  
“experiments”.  
Which one has the  
strongest main  
versus random  
effect?

High temperature



Average  
within  
treatment =  
40g

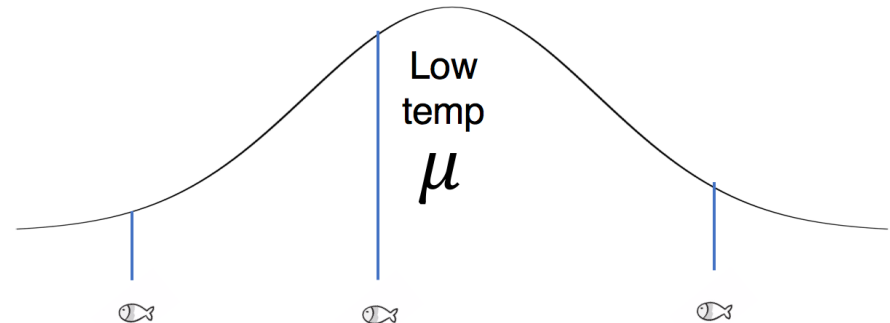
Tank

Tank

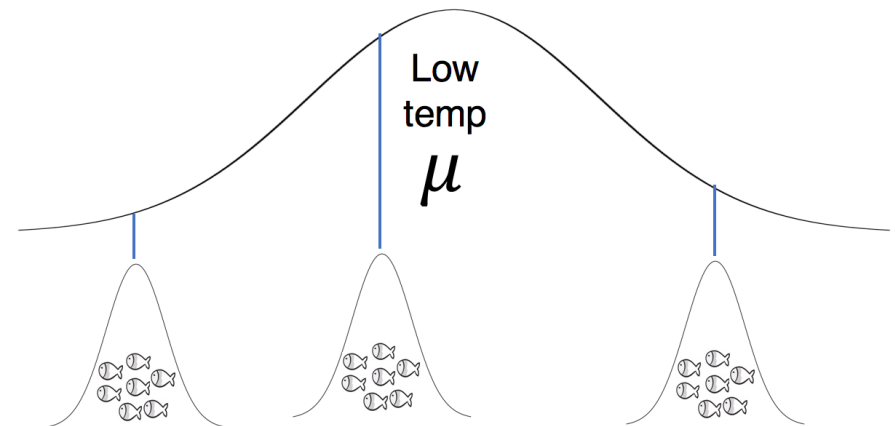
# Hierarchical (clustered) sampling

Hierarchical (clustered) sampling is extremely common, rather than simple random sampling, mainly as a means of saving resources when, for example, the population is spread out, and the researcher cannot sample from everywhere. However, observations in the same cluster are likely to be somewhat more similar to one another.

Non-hierarchical random sample (fixed)



Hierarchical (random)



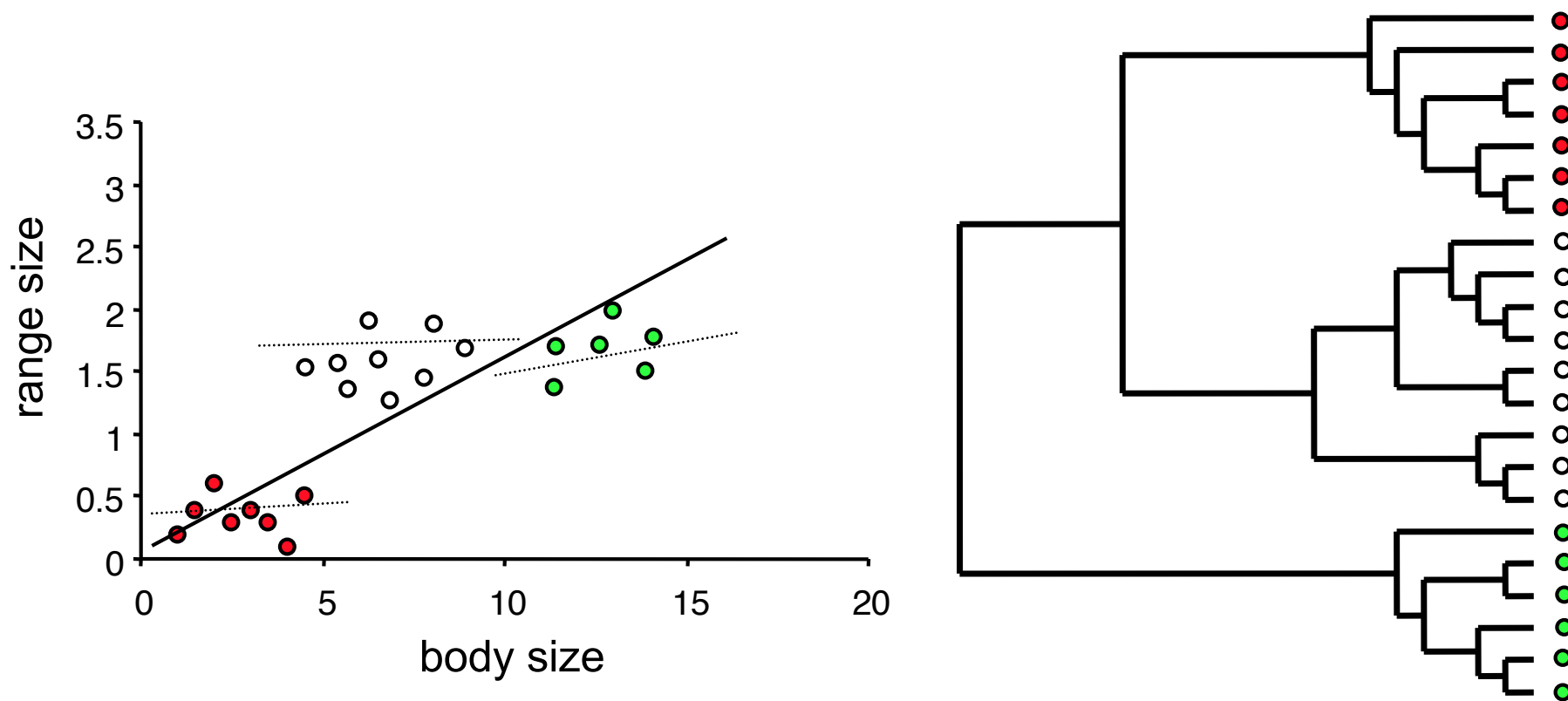
adapted from Gene Shackman



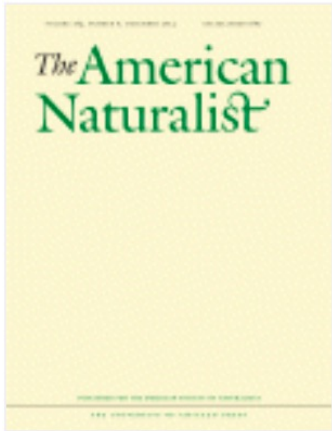
## Hierarchical (clustered) sampling

As a result, in a clustered sample selecting, an additional observation from the same cluster adds less new information than would a completely independent selection. As such, the sample is not as variable a random sample would be, so that the effective sample size is reduced. The loss of effective sample size by using clustered sampling, instead of simple random sampling, is called **design effect**.

Design effect under the lenses of a common  
ecological problem – phylogenetic variation  
How many independent data points do we have?



The loss of effective sample size by using clustered sampling, instead of simple random sampling, is called **design effect**. Perhaps of 23 species we may have only “3 independent observations” (i.e., design effect = 3).



## JOURNAL ARTICLE

### Phylogenies and the Comparative Method

Joseph Felsenstein

*The American Naturalist*

Vol. 125, No. 1 (Jan., 1985), pp. 1-15

Published by: [The University of Chicago Press](https://www.jstor.org/stable/2461605) for [The American Society of Naturalists](https://www.jstor.org/stable/2461605)

<https://www.jstor.org/stable/2461605>

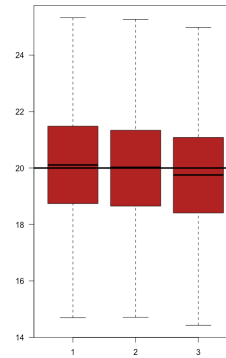
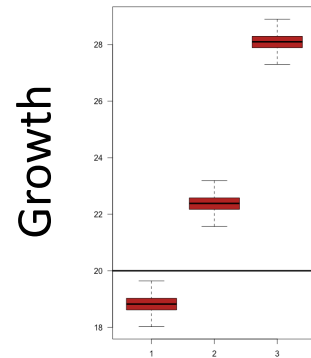
Page Count: 15

**The loss of effective sample size** by using clustered sampling, instead of simple random sampling, is called **design effect**. Perhaps of 23 species we may have only “3 independent observations”.

**INCREASED TYPE I ERROR IF KEPT AT 23 species (i.e., wrongly considering too many independent observations) AND LOSS OF POWER** if only “3 species” is used.

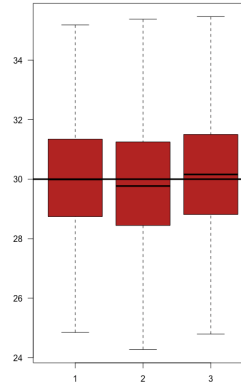
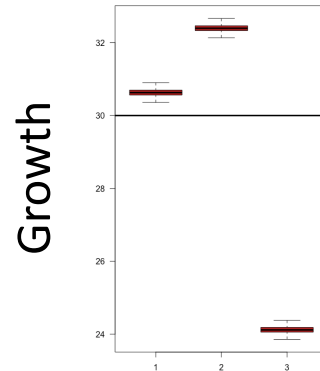
# In which case is the design effect larger? Left or right column?

Low temperature



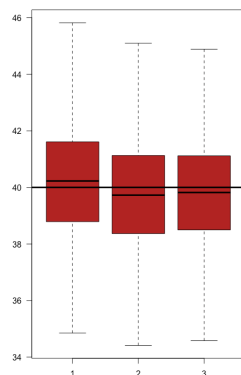
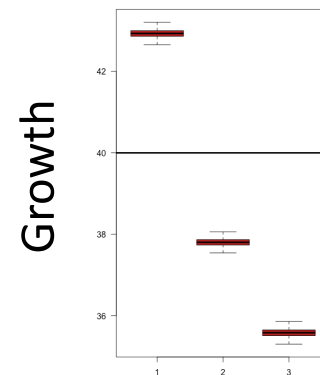
Average  
within  
treatment =  
20g

Intermediate  
temperature



Average  
within  
treatment =  
30g

High temperature



Average  
within  
treatment =  
40g



Tank

Tank

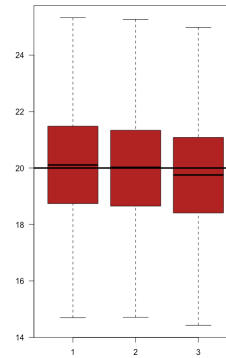
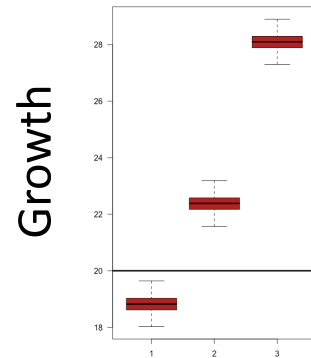
## Hierarchical (clustered) sampling

The **design effect** is a correction factor that is used to adjust the sample size based on clustered sampling. This accounts for the loss of information inherent in the clustered design and is used when estimating random effects.

Once the design effect is calculated, the sample size calculated for a standard design can be adjusted accordingly (i.e., degrees of freedom are corrected). As such, the statistical power may change according to the design effect.

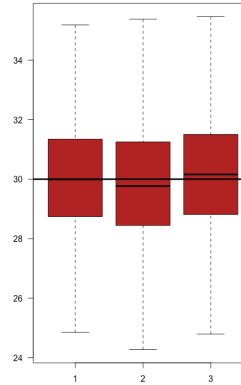
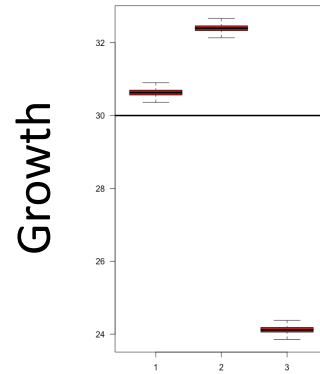
In which case is the statistical power greater? Left or right column?

Low temperature



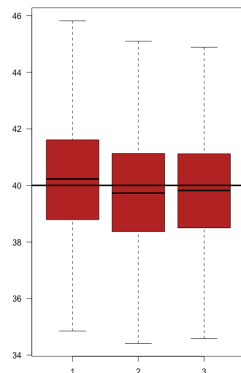
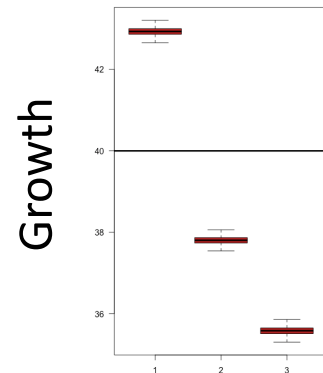
Average  
within  
treatment =  
20g

Intermediate  
temperature



Average  
within  
treatment =  
30g

High temperature



Average  
within  
treatment =  
40g



Tank

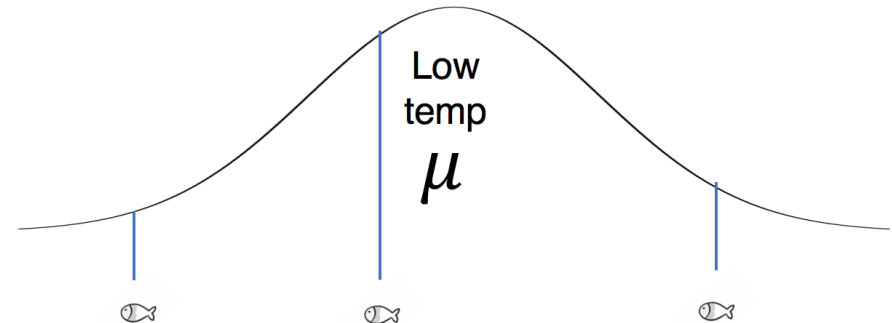
Tank



# Hierarchical (clustered) sampling

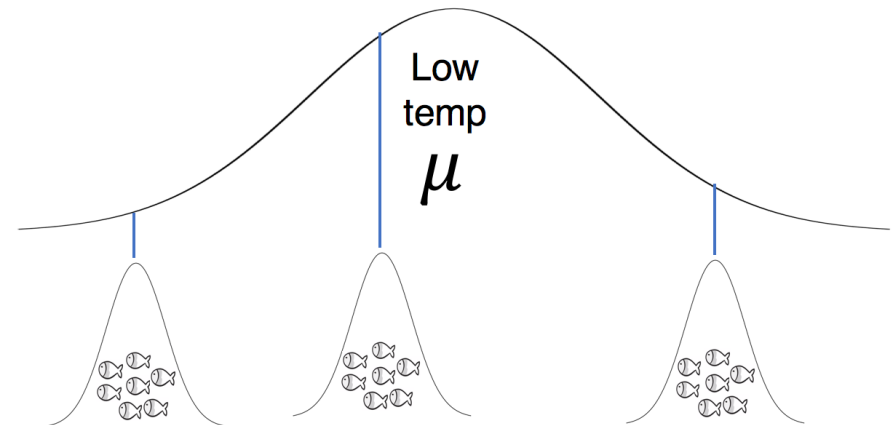
Hierarchical (clustered) sampling is extremely common, rather than simple random sampling, mainly as a means of saving resources when, for example, the population is spread out, and the researcher cannot sample from everywhere. However, observations in the same cluster are likely to be somewhat more similar to one another. decreasing effect size.

Non-hierarchical random sample (fixed)



Which design is statistically more powerful? It depends on the design effect of the data.

Hierarchical (random)



adapted from Gene Shackman

Intraclass correlation as a way to understand why  
to use a random effect and how much “random”  
has an “effect”



## Hierarchical (clustered) sampling – intraclass correlation & design effect (one extreme example)

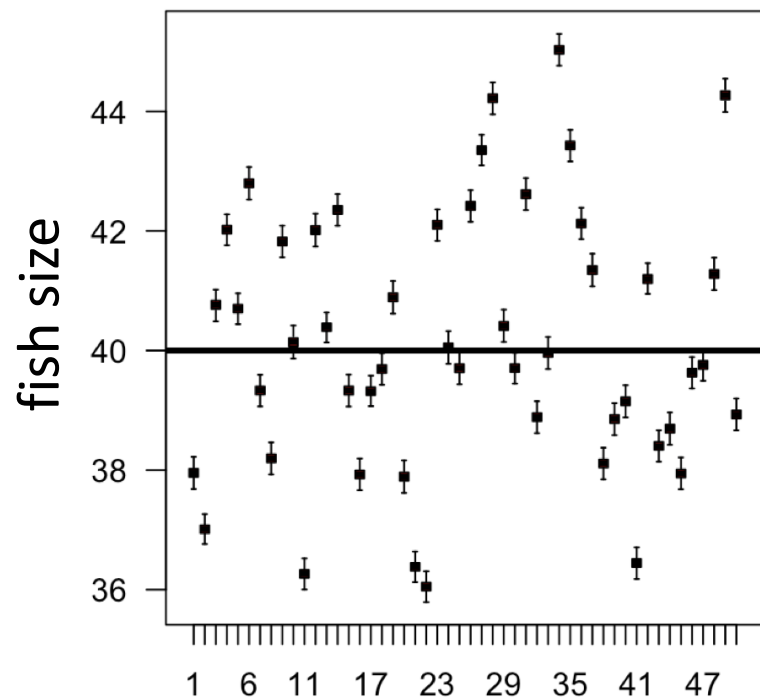
- Consider a study that wants to estimate fish size between two regions in Quebec (Laurentians and Eastern Townships).
- Draw a random sample of 50 lakes in each region.
- Randomly sample 10 individuals in each lake ( $n = 10$ ).

One fixed factor = region (these two regions cannot change).

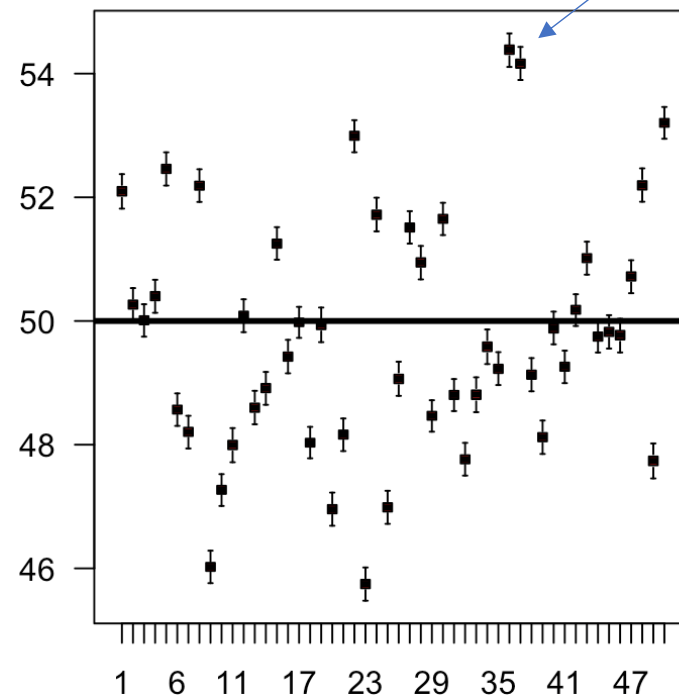
One random factor = lakes (because they are more likely to be similar within regions and they are not crossed between regions, i.e., different lakes are used).

- Consider a study that wants to estimate fish size between two regions in Quebec (Laurentians and Eastern Townships).
- Draw a random sample of 50 lakes in each region.
- Randomly sample 10 individuals in each lake ( $n = 10$ ).

*Each confidence interval represents variation in size of 10 fish*



Lakes (Laurentians)

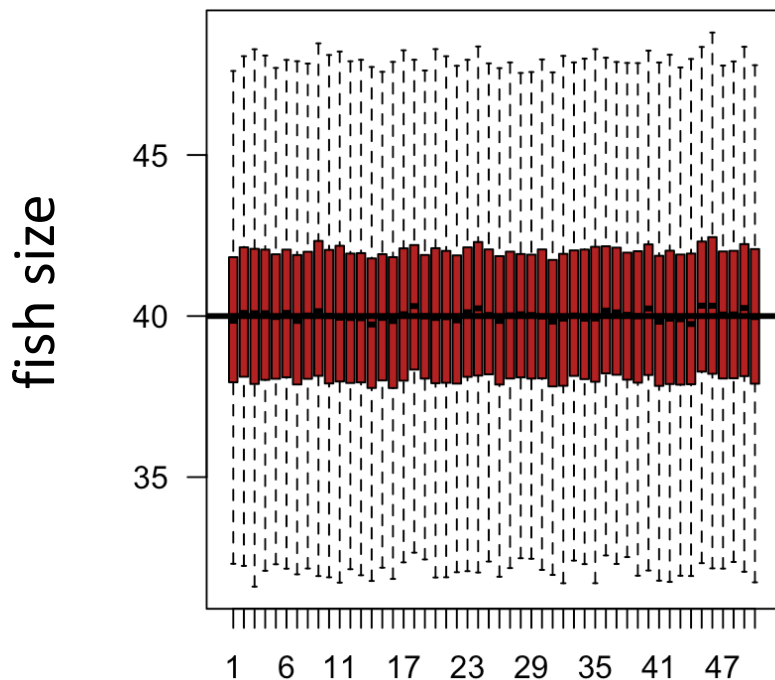


Lakes (Eastern Townships)

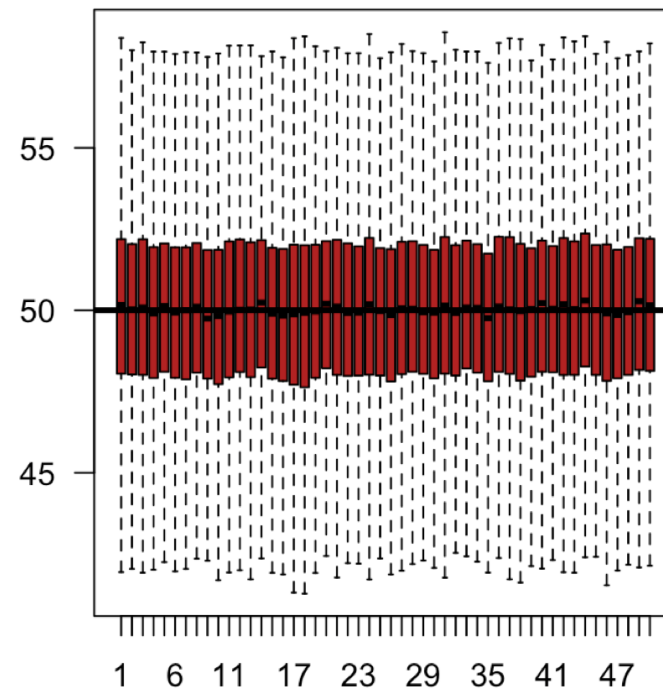
Very high ( $\sim 1$ ) intraclass correlation here because individuals are very similar within lakes than among lakes; so, a random effect would be important.

- Consider a study that wishes to estimate fish size between two regions in Quebec (Laurentians and Eastern Townships).
  - Draw a random sample of 50 lakes in each region.
  - Randomly sample 10 individuals in each lake ( $n = 10$ ).
- 
- Assume for the sake of discussion that all individuals within each lake had the exact same size but size differed between each of the lakes, then  
*intraclass correlation = 1 and a design effect =  $1 + 1(50 - 1) = 50$ .*
- 
- In this case, we have started our sample with 500 individuals across lakes, but the “design” can only “use” 50 values (“observations”) in the analysis because all individuals were too similar within lakes! So, to increase the degrees of freedom, we would need to sample now 500 lakes per region instead! So, this design effect reduced the statistical power of the ANOVA.

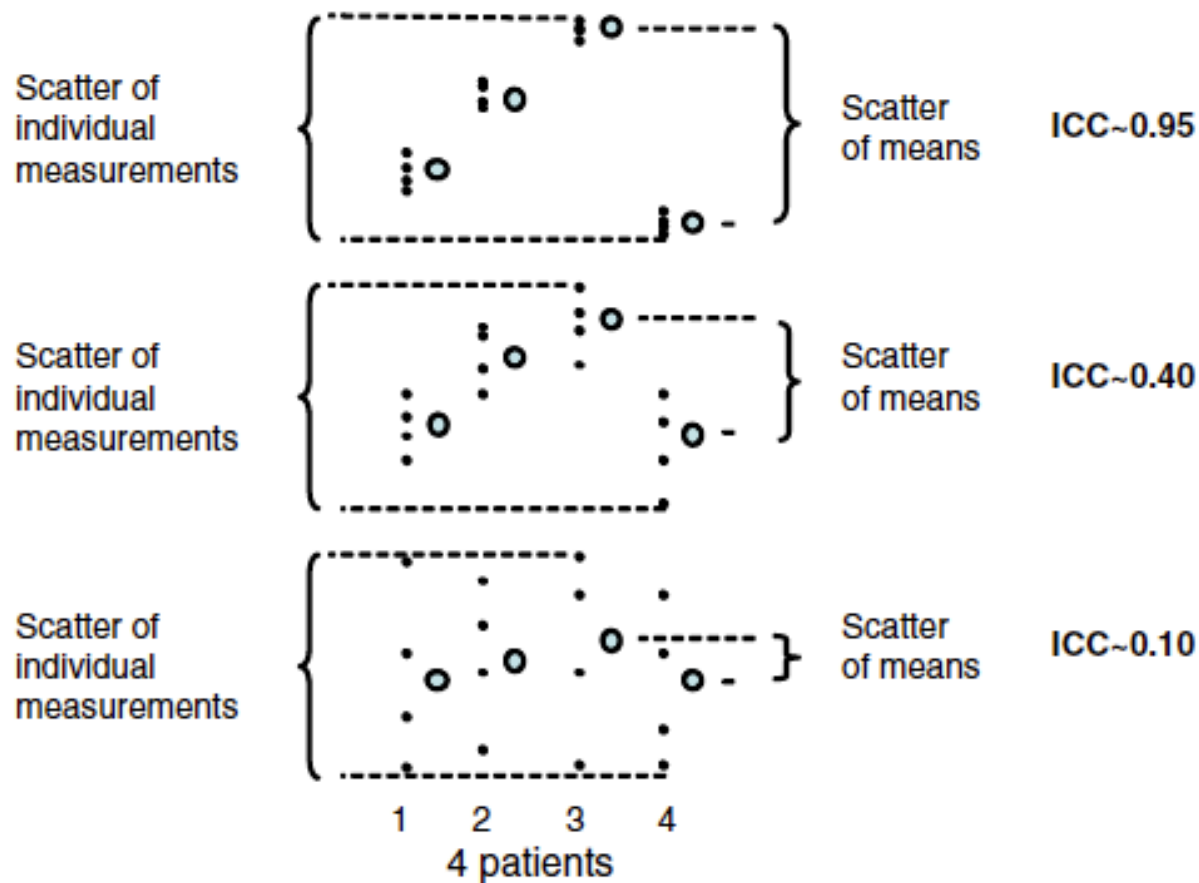
- Consider now these data (two other regions): If variation in size *within* each lake is the same for all lakes, then  
*intraclass correlation = 0* and *what we call design effect = 1 + 0(50 - 1) = 1*.
- In this extreme case, each additional lake adds no new information about the fish size in each region.
- Only surveying one lake would give us the same information (with the same standard error) about fish size as we get from surveying 50 lakes. So, to get the same degrees of freedom we only need to sample now 500 fish in a single lake per region instead of 10 individuals across 50 lakes!



Lakes (Outaouais)



Lakes (Abitibi)



intraclass correlation  
coefficient (ICC) or rho

Schematic demonstrating intraclass correlation coefficient (ICC) as a measure of reproducibility. 4 patients each have measurements made 4 times (small dots) with each patient also summarised by an individual average (large dot). In the top panel, there is little within-patient scatter, and therefore the ratio of variance of mean (large dots) to the variance of the raw data (small dots) is almost 1, so  $ICC \approx 1$ . In the middle panel, the ICC is lower. In the bottom panel, within-patient scatter is large, and the means much less varied than the raw data, so ICC is low.

Extracted from Moraldo et al. (2013); International Journal of Cardiology, 166:688-695.

Mixed models mix random and fixed effects and allows estimating conducting statistical testing (inference) via proper estimation of design effects for hierarchical (clustered) sampling! It also affects parameter estimation (e.g., Simpson's paradox)



## Why consider a mixed-model?

Some factors you may be able to control (fixed) and others you won't (random)

- (i) Models using random effects are important for inference when analyzing data that exhibit non-independence (hierarchical structure).
- (ii) Random effects provide a unifying statistical framework for models that might otherwise seem unrelated, for example, time-series models for populations, spatial models, genetics models, and models for variation among individuals;
- (iii) Models that include random effects are increasingly easy to build and customize for specific problems using publicly available modelling tools and software.

# Time for **reading**

The PeerJ logo is a blue square with the word "PeerJ" in white. The "P" is large and bold, and the "J" has a distinctive shape with a curved tail.

PeerJ

## **A brief introduction to mixed effects modelling and multi-model inference in ecology**

Xavier A. Harrison<sup>1</sup>, Lynda Donaldson<sup>2,3</sup>, Maria Eugenia Correa-Cano<sup>2</sup>, Julian Evans<sup>4,5</sup>, David N. Fisher<sup>4,6</sup>, Cecily E.D. Goodwin<sup>2</sup>, Beth S. Robinson<sup>2,7</sup>, David J. Hodgson<sup>4</sup> and Richard Inger<sup>2,4</sup>

Mixed models mixes random and fixed effects!

(next lecture)