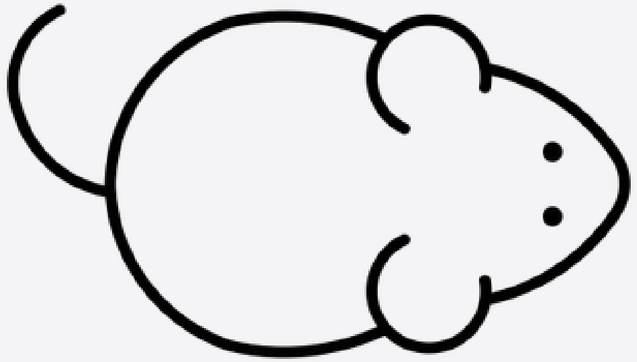
MICE IN CAGES

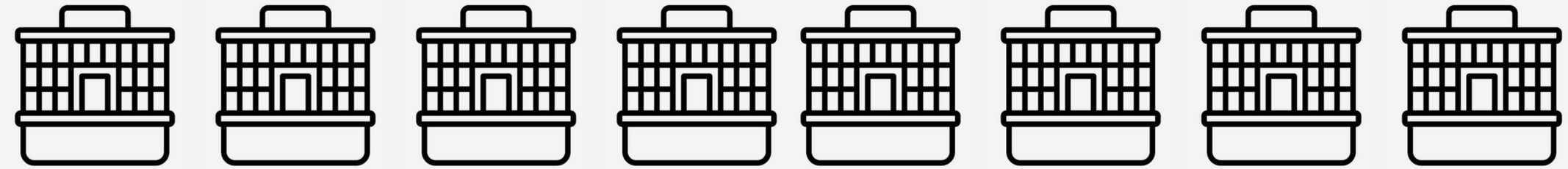
How ignoring nested designs can ruin your data analysis



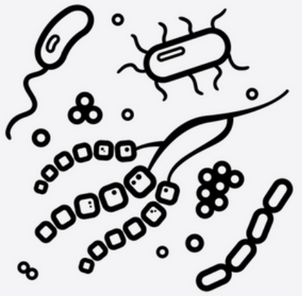
Esma Boudemagh
14.08.2024

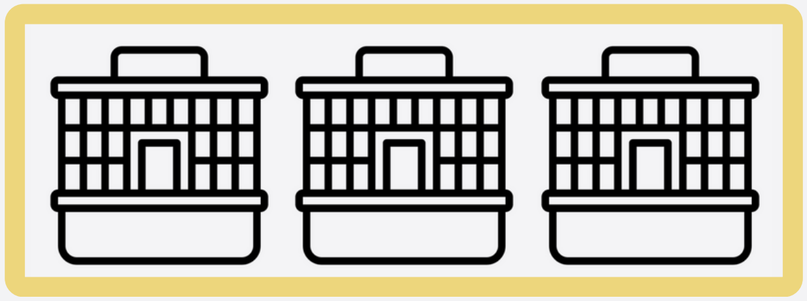
Modelling project
S. Bergmann, F. Schütz

Are mice really clones?



Are mice really clones?





lighting differences

handling differences

microbiota similarities

THE MICE WEIGHTS DATASET HAS A NESTED DESIGN

19 cages

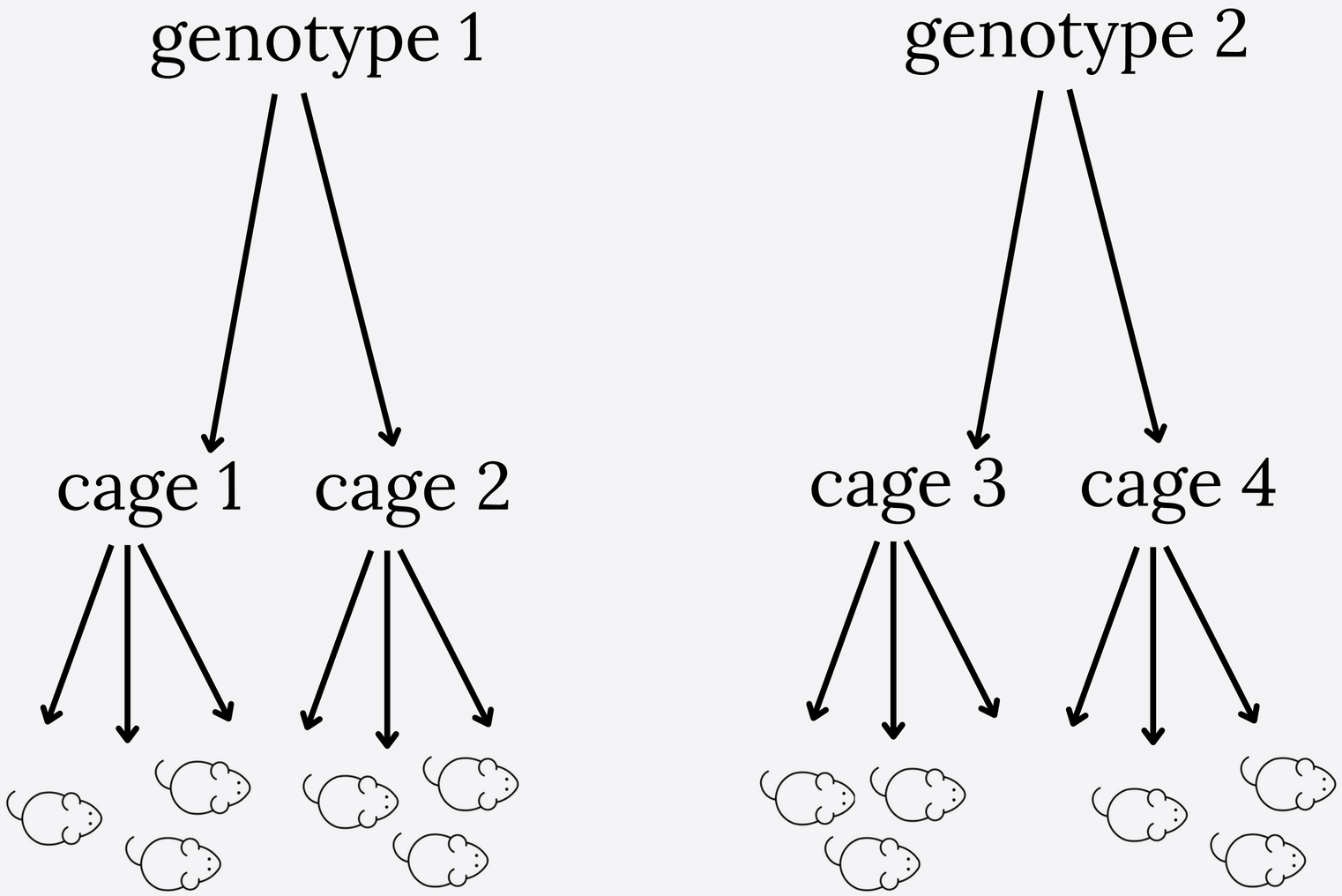
1-8 mice per cage

Cage <fctr>	poids <dbl>	Genotype <fctr>
584	28.8	+/+ +/+
584	30.5	+/+ +/+
584	28.8	+/+ +/+
584	31.7	+/+ +/+
605	32.5	+/+ +/+
605	29.3	+/+ +/+
605	30.5	+/+ +/+
605	27.7	+/+ +/+
605	28.0	+/+ +/+

...

5 genotypes possible

THE MICE WEIGHTS DATASET HAS A NESTED DESIGN



THE MICE WEIGHTS DATASET HAS A NESTED DESIGN

the effect of the cage is nested into the effect of the genotype

THE MICE WEIGHTS DATASET HAS A NESTED DESIGN

the effect of the cage is nested into the effect of the genotype

how do we take it into account?

STATISTICAL MODELS

one-way ANOVA
hierarchical ANOVA
mixed model

STATISTICAL MODELS

one-way ANOVA

only fixed pre-determined effects
-> risk of increased false positives
-> between-cages variance not efficient

STATISTICAL MODELS

hierarchical ANOVA and mixed models

can handle fixed and random effects
-> cage effect can be added as a random effect

PROJECT OUTLINE

1. QUANTIFICATION OF CAGE EFFECT
2. SIMULATIONS FOR DATA ANALYSIS
3. SIMULATIONS FOR EXPERIMENTAL DESIGN

1. QUANTIFICATION OF CAGE EFFECT

does our data actually have a cage effect?

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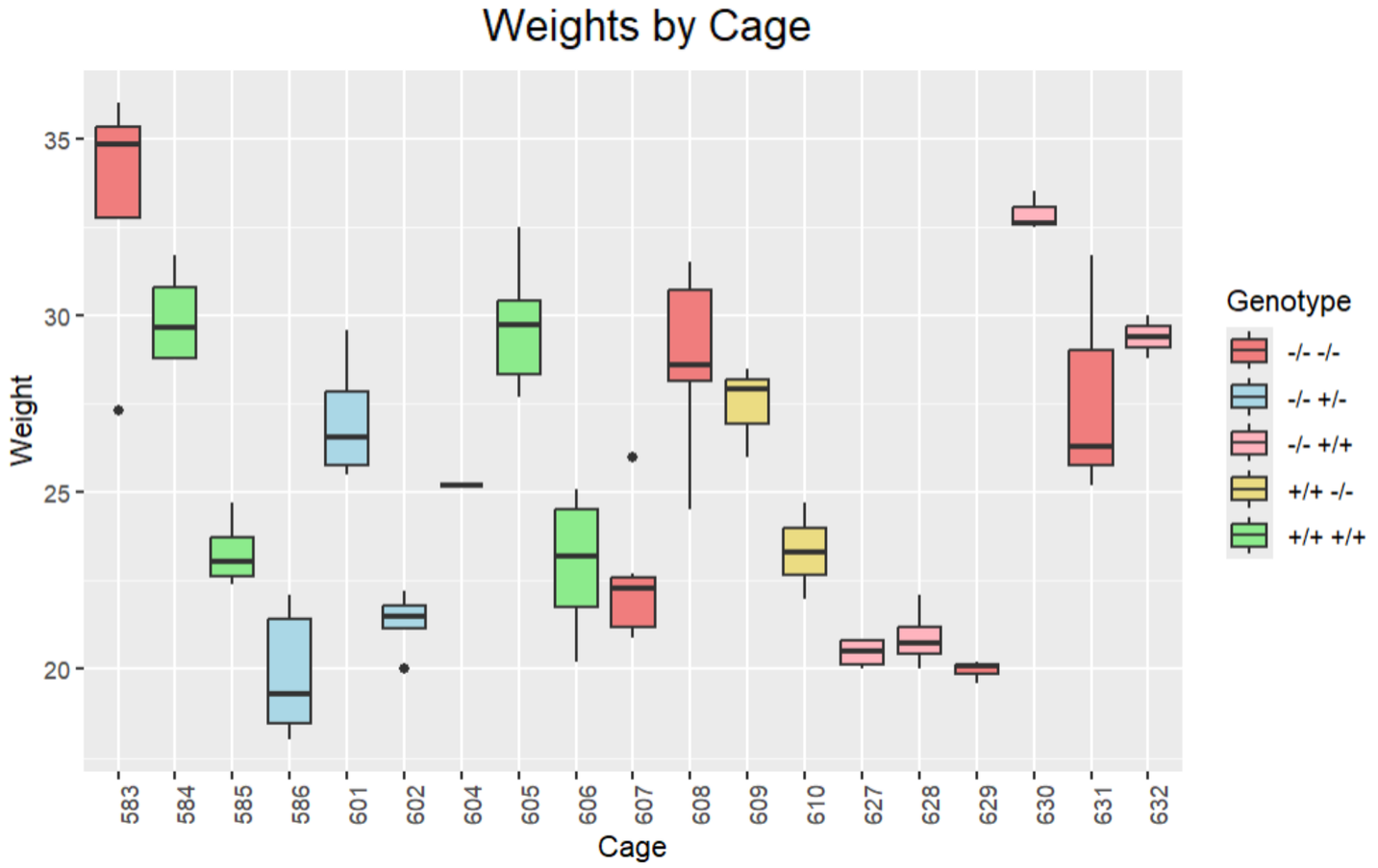

1. QUANTIFICATION OF CAGE EFFECT

does our data actually have a cage effect? yes

residuals of one-way ANOVA > residuals of hierarchical ANOVA

```
Analysis of Variance Table

Response: poids
           Df  Sum Sq Mean Sq F value  Pr(>F)
Residuals 83 1554.94  18.734
```

```
Analysis of Variance Table

Response: poids
           Df  Sum Sq Mean Sq F value    Pr(>F)
Residuals  69  224.87   3.259
```

the model that takes into account cage effect explains more of the variance than the simpler one

1. QUANTIFICATION OF CAGE EFFECT

standard deviation due to the cage effect ~= **8.5g**
(average mouse weight of the dataset ~25g)

-> large effect

2. SIMULATIONS

2 groups, one control and one with the effect of a treatment

24 mice in total (12 per group)

cage effect standard deviation is 8.5 grams

2. SIMULATIONS FOR DATA ANALYSIS

3 cages 4 mice, 1000 simulations
cage effect bigger than treatment effect

false positive rates
treatment effect = 0
cage effect = 8.5

one-way: 43.5%
hierarchical: 5%
mixed: 13%

power
treatment effect = 5
cage effect = 8.5

one-way : 50.3%
hierarchical : 44.4%
mixed : 17.6%

2. SIMULATIONS

3 cages 4 mice, 1000 simulations
treatment effect bigger than cage effect

power
treatment effect = 10
cage effect = 8.5

mixed : 39.3%

3. SIMULATIONS FOR EXPERIMENTAL DESIGN

changing number of cages
cage effect bigger than treatment effect

false positive rates
treatment effect = 0
cage effect = 8.5

4 cages 3 mice: 9.8%
6 cages 2 mice: 8.4%

power
treatment effect = 5
cage effect = 8.5

4 cages 3 mice: 16.1%
6 cages 2 mice: 23.6%

3. SIMULATIONS FOR EXPERIMENTAL DESIGN

changing number of cages
treatment effect bigger than cage effect

false positive rates
treatment effect = 0
cage effect = 8.5

4 cages 3 mice: 9.8%
6 cages 2 mice: 8.4%

power
treatment effect = 10
cage effect = 8.5

4 cages 3 mice: 45.5%
6 cages 2 mice: 52%

3. SIMULATIONS FOR EXPERIMENTAL DESIGN

changing number of cages
treatment effect bigger than cage effect

highest power obtained

false positive rates
treatment effect = 0
cage effect = 8.5

4 cages 3 mice: 9.8%
6 cages 2 mice: 8.4%

power
treatment effect = 10
cage effect = 8.5

4 cages 3 mice: 45.5%
6 cages 2 mice: 52%

3. SIMULATIONS FOR EXPERIMENTAL DESIGN

changing number of cages
treatment effect bigger than cage effect

false positive rates
treatment effect = 0
cage effect = 8.5

2 cages 6 mice: 20.6%

power
treatment effect = 10
cage effect = 8.5

2 cages 6 mice: 40.2%

SUMMARY OF RESULTS

unexpected results for false positives and power

-> expected higher power with increased number of cages and
lower false positive rates for mixed models and hierarchical models

-> number of cages and mice per cage too small?

LIMITATIONS

simulations might be too simple compared to the reality (2 treatments vs 5 genotypes)

THANK YOU
QUESTIONS?