Experimental designs and data analysis in R

Learning Objectives

Design, analyze, and interpret results from a **factorial experiment**

Identify **focal** and **moderator** treatment effects

Plan **blocks**, **replication**, and **interspersion** to maximize efficiency

Create and interpret statistical conclusions

Manage data in spreadsheets

Use R to inspect, plot, and analyze data

Williams

BS Biology

Plasticity in tadpoles

UC DAVIS
UNIVERSITY OF CALIFORNIA

Postdoc, Professor

Plant development, evolution, breeding

Duke
UNIVERSITY

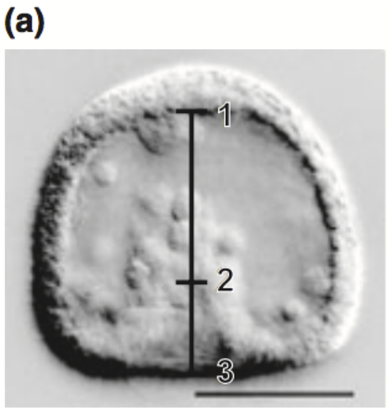
PhD Biology

Sea urchin development

MS Statistics

Quantitative genetics

Big Data



View on GitHub

PLS 205

Lectures and materials for PLS 205: Experimental Design and Analysis at UC Davis

https://deruncie.github.io/PLS205_course/Course_content.html

Week	Topics	Lectures	Labs
1	Introduction, Estimating treatment effects	Lecture 1 Lecture 2	Lab 1
2	Standard Errors and Confidence Intervals; Indirect estimates	Lecture 3 Lecture 4	Lab 2
3	Analysis of Design 1 - Indirect estimation; Analysis of Design 3 - Subsamples; Comparisons of Designs 1-3	Lecture 5 Lecture 6	Lab 3
4	When treatments have >2 levels; ANOVA	Lecture 7 Lecture 8	Lab 4
5	Data Transformations; Replication	Lecture 9 Lecture 10	Lab 5
6	Introduction to Factorials; More factorials	Lecture 11 Lecture 12	Lab 6
7	Continuation of Factorials, intro to the RCBD; Generalized RCBD	Lecture 13 Lecture 14	Lab 7
8	RCBD with replicates; Incomplete Blocks	Lecture 15 Lecture 16	Lab 8
9	Split Plot designs; More split plots!	Lecture 17 Lecture 18	Lab 9
10	More Split Plots; Review	Lecture 19 Lecture 20	

Outline

Morning

Research Questions

- Define and measure **treatment effects**
- Load and inspect data in R
- Fit models and extract summaries
- Data transformations

Afternoon

Experimental Designs

- Define Experimental Units and Blocks
- Discuss confidence, replication, and power
- Use Design Tables to set up model statements
- Design and describe an experiment

Evening

Analyze data

- Interpret an experimental description
- Analyze data
- Produce a report
- Critique the experiment

Let’s run an experiment!

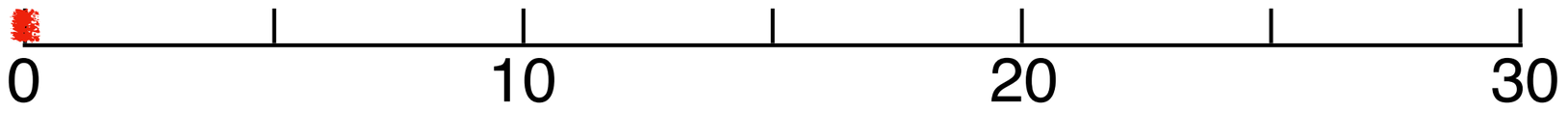
Research Question: Does standing affect a person’s pulse?

Experimental Design:

Assign each person to **stand** or **sit**. Apply the treatments. Measure pulse for 30s.

On “Ready”, get in position and find your pulse. On “Go”, start counting…

Ready . . . **Set . . .** **Go!** **Stop!**



Pulse (beats per minute) = count x 2

Write a statement about the results

What conclusions can you draw?

Include:

Statements about **size** and **direction** of the treatment effect

Statements about **subjects** and **population**

Statements about **Confidence / Certainty** about the conclusions

Standing **increased** pulse relative to sitting by XX bpm (95% CI: XX-XX bpm) among graduate students on the 3rd day of class at CSHL

Standing vs sitting had a **significant** effect on pulse ($\alpha < 0.05$) …

There was moderate evidence of an effect of standing vs sitting (p = 0.0023) …

Guidelines

Make statements about **treatment effects**

Treatments are comparisons between **2 levels** Sitting vs Standing Mutant vs WT

Not Significant doesn't mean **No Effect**

We can conclude that an **effect > 0**, but we cannot conclude that there is **no effect**

$p > 0.05$ may be *not significant*, but this doesn't mean that there is no effect

Look at Confidence Intervals as a range of **plausible values**

The p-value reported by *R* may not be the p-value for your **Research Question**

Often we need to **combine p-values** to address the real question

Do any genes change in expression?

Does geneA affect flowering in Long Days or Short Days?

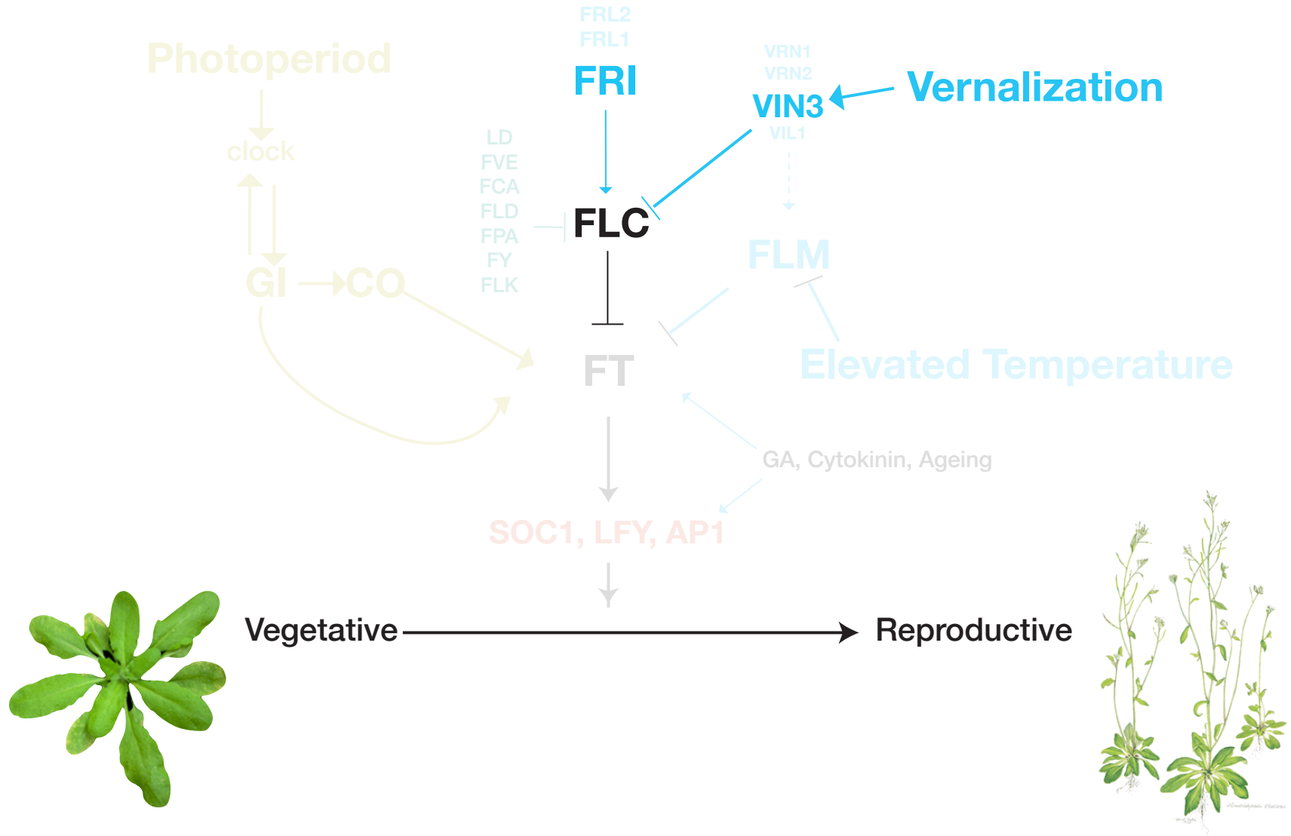
New Phytologist

Full paper | Free Access

Fluctuating, warm temperatures decrease the effect of a key floral repressor on flowering time in *Arabidopsis thaliana*

Liana T. Burghardt, Daniel E. Runcie, Amity M. Wilczek, Martha D. Cooper, Judith L. Roe, Stephen M. Welch, Johanna Schmitt

First published: 17 December 2015 | https://doi.org/10.1111/nph.13799 | Citations: 32



What is this study about?

Effect of FRIGIDA (FRI) on flowering time

in *Arabidopsis thaliana*

focal effect *FRI* vs *fri*

How fluctuating, warm temperatures **alter** the effect of FRI on flowering time

How two other genes (*FLC* and *VIN3*) interact with *FRI*

moderator effects

fluctuating vs constant

\+ vs - vernalization

FLC vs *flc*

VIN3 vs *vin3*

Focal treatment

The essential perturbation of study

Col-0 genotype has a non-functional *FRI* allele

What is the effect on flowering time of re-activating it?

Compare *Col FRI* to *Col-0*

Key concept:

There is never **one effect** of a treatment

What is the average effect?

How consistent is the effect?

What will the effect be for a specific plant?

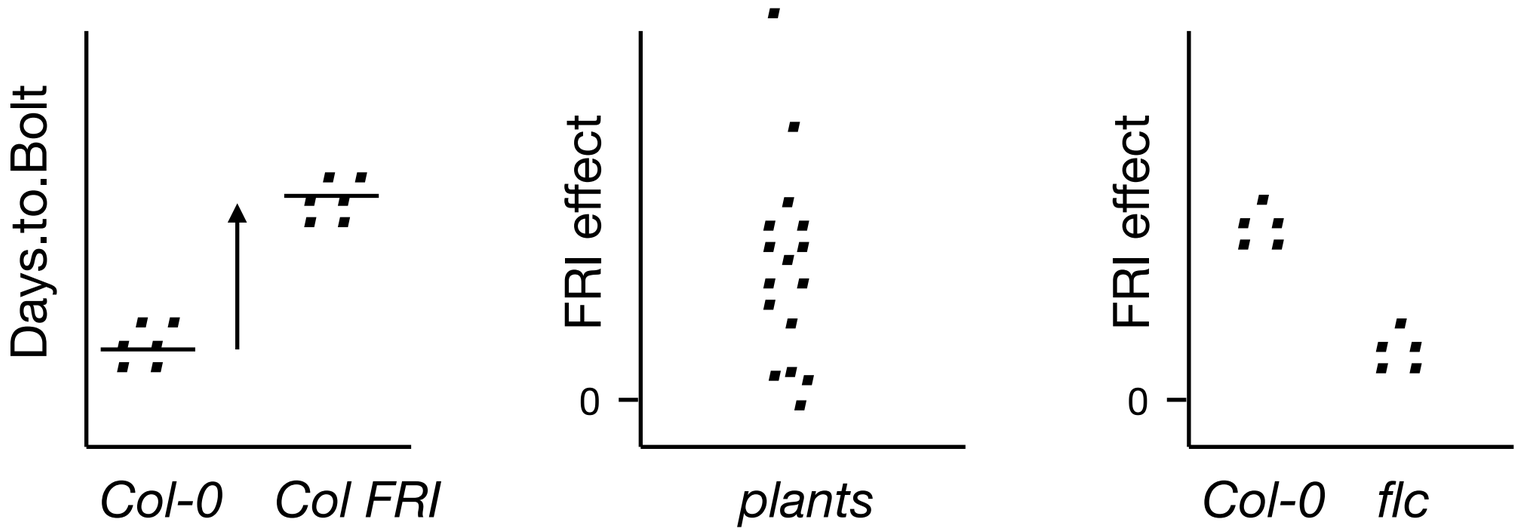
What factors change the effect?

Factorial experiment

2+ treatments

focal and moderator

Measure the effect of the moderator treatment on the effect of the focal treatment



environments?

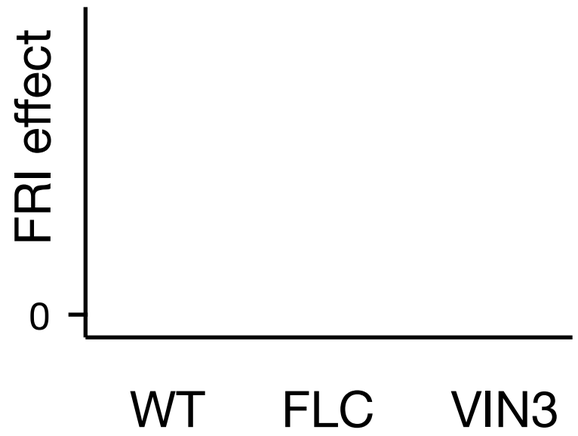
genetic backgrounds?

random?

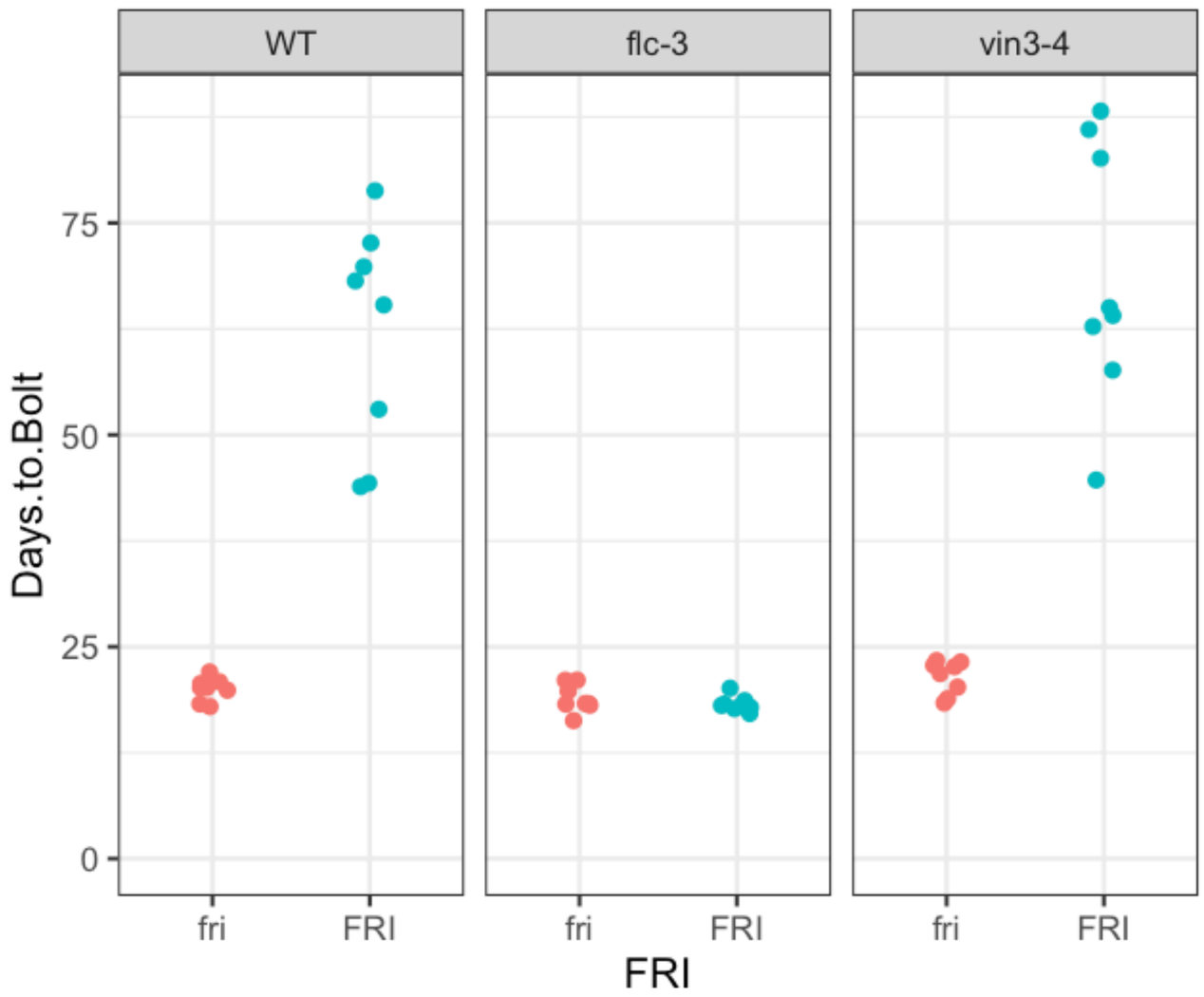
Analysis 1: How do FLC and VIN3 modify the effect of FRI?

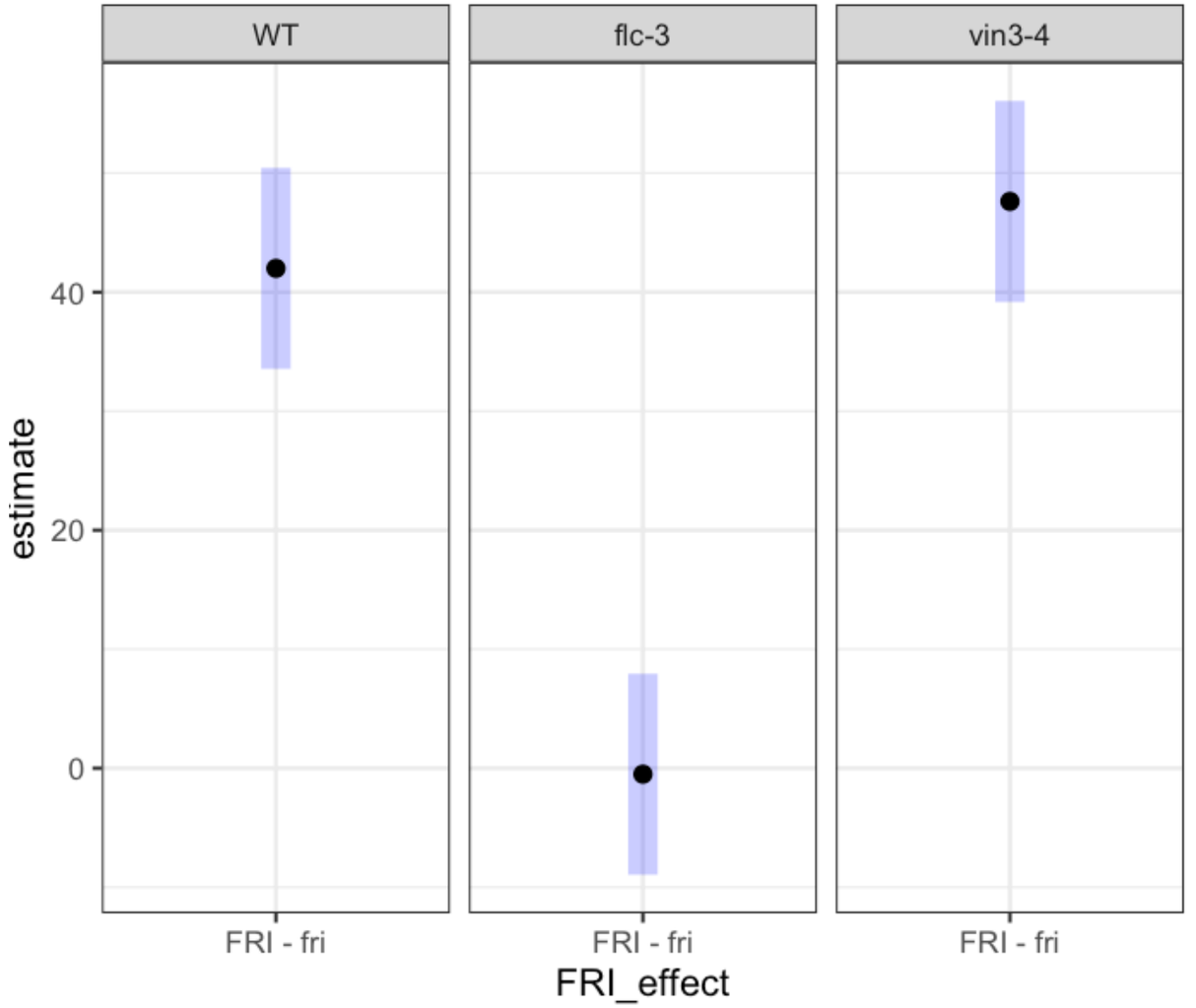
The essential perturbation of study

		Mutant		
		WT	FLC	VIN3
FRI	fri	Col	flc-3	vin3-4
	FRI	Col FRI	flc-3 FRI	vin3-4 FRI



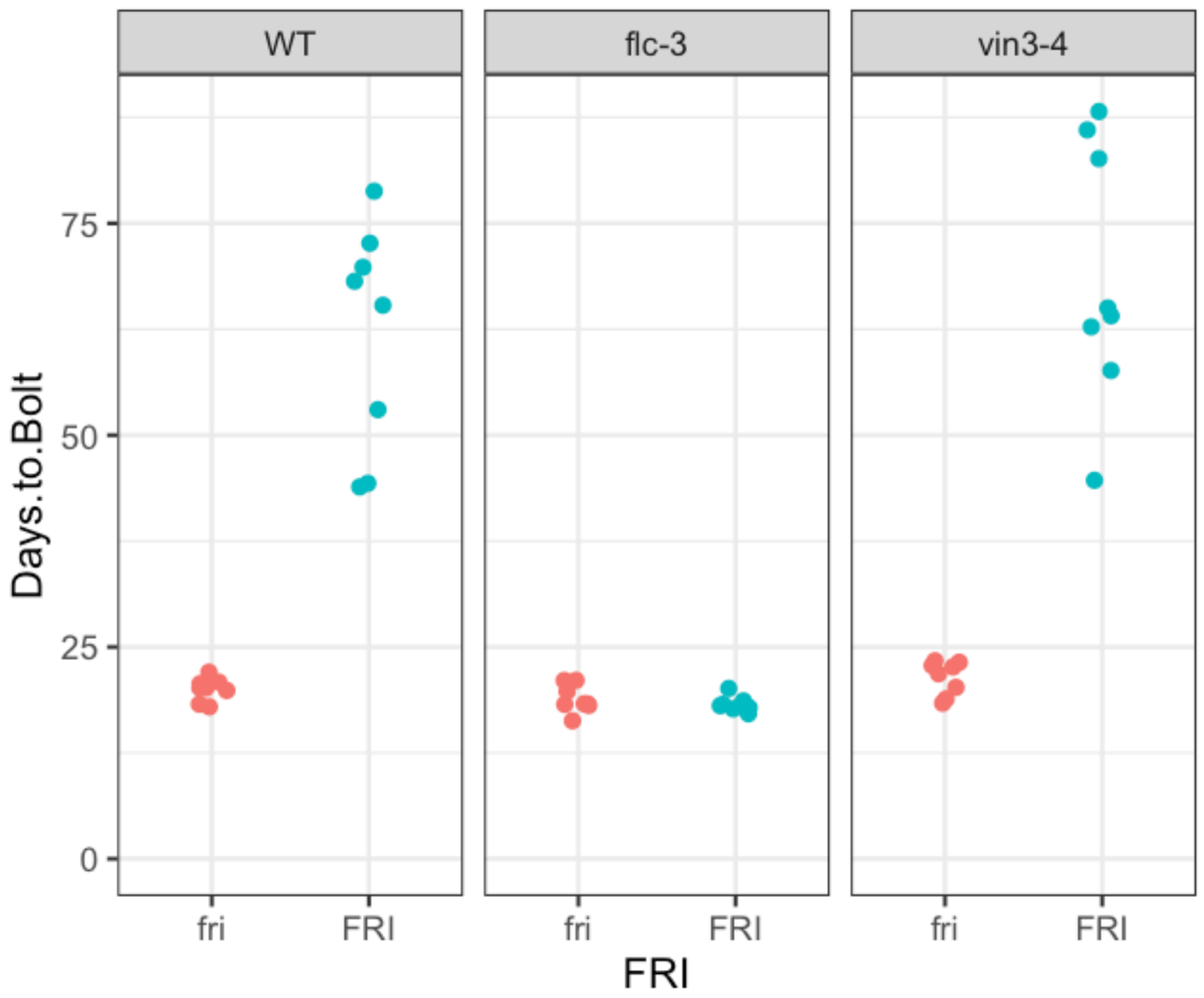
Analysis 1: Summary

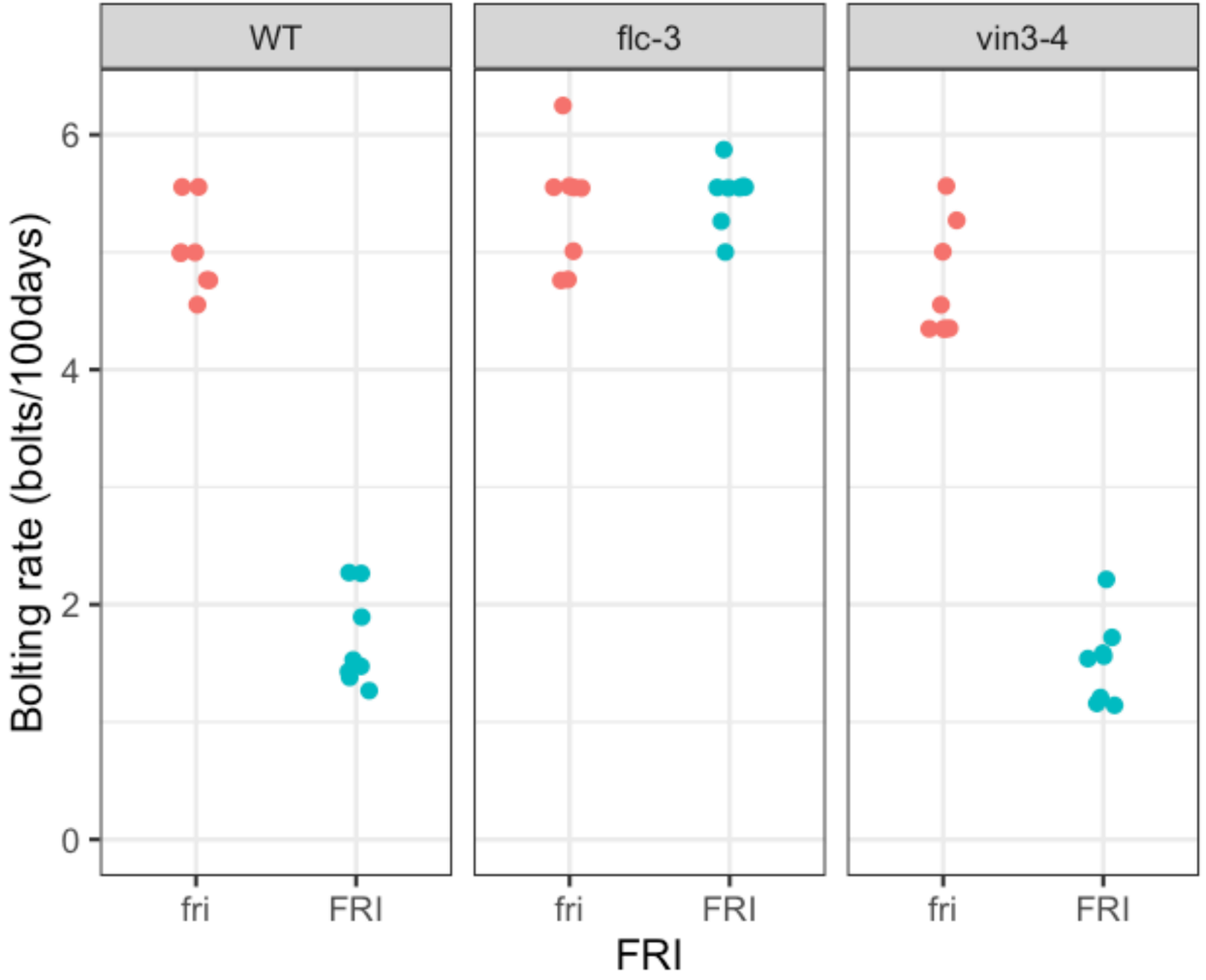




Are these **estimates** good summaries of the FRI effect?

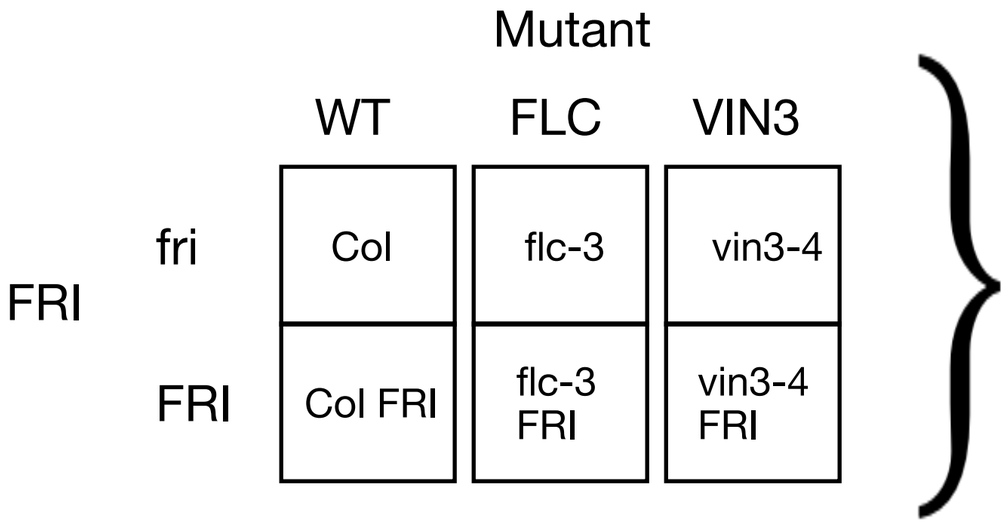
Data transformations - 1/Days.to.Bolt





On this scale, the FRI effects are more similarly sized among replicates

Analysis 2 - FRI effects in different conditions



22C + Constant Temp + No-Vernalization

22C + Constant Temp + Vernalization

22C + Variable Temps + No-Vernalization

22C + Variable Temps + Vernalization

2 x 3 x 4 factorial

24 treatment combinations

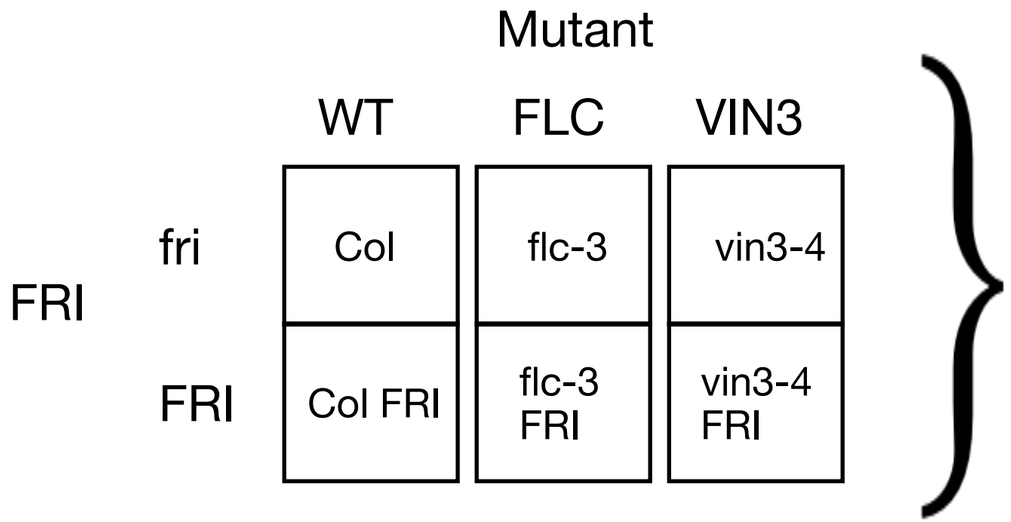
What can we learn?

focal effect: (FRI - fri) on Bolting_rate

moderator 1: Mutant (flc-3 - WT) or (vin3-4 - WT)

moderator 2: Env effect on mutant's effect on FRI effect

Analysis 2 - FRI effects in different conditions



22C + Constant Temp + No-Vernalization

22C + Constant Temp + Vernalization

22C + Variable Temps + No-Vernalization

22C + Variable Temps + Vernalization

Plan:

1. Measure Mutant effects on FRI effect in each condition

2. Compare these effects

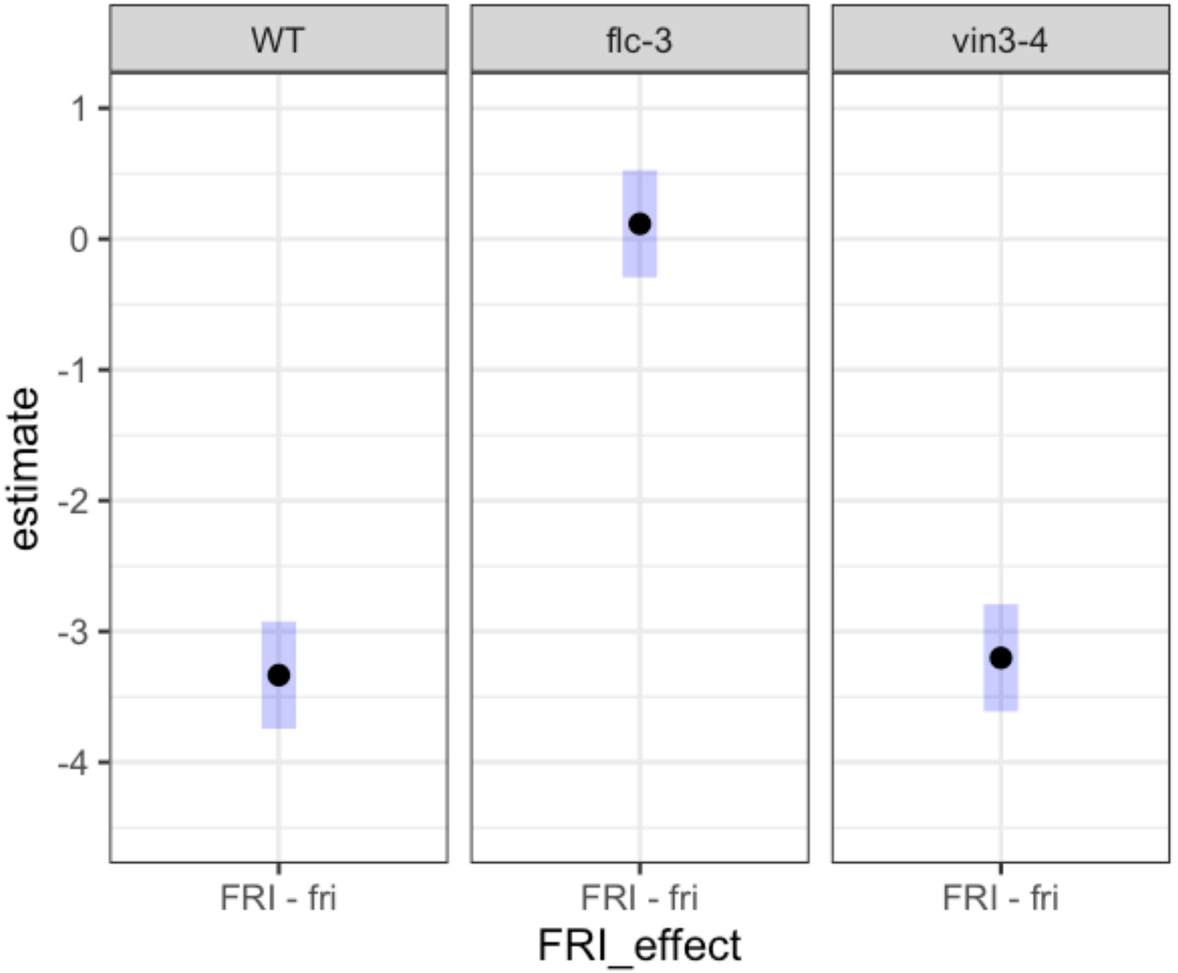
focal effect: (FRI - fri) on Bolting_rate

moderator 1: Mutant (flc-3 - WT) or (vin3-4 - WT)

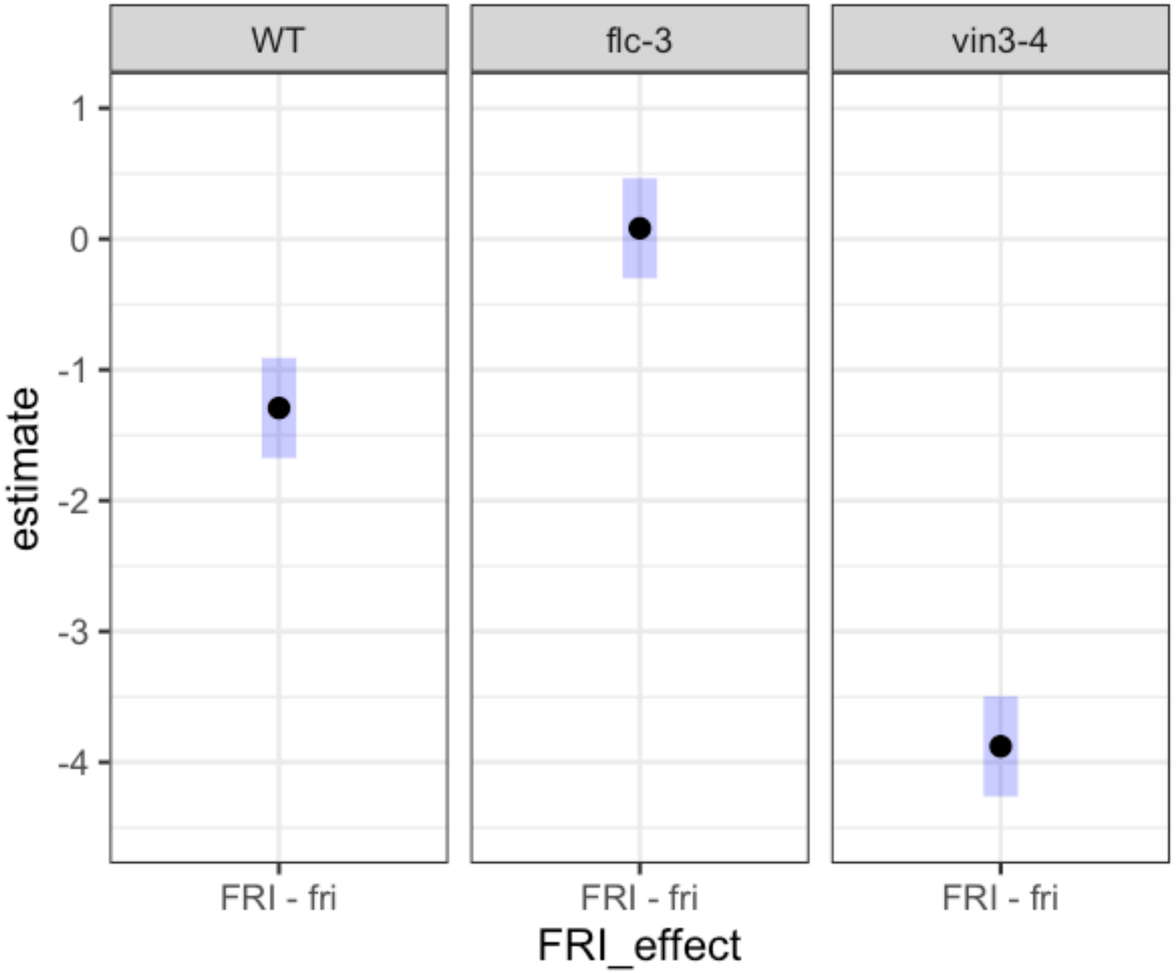
moderator 2: Env effect on mutant's effect on FRI effect

Analysis 2 - FRI effects in different conditions

22C + Constant Temp + No-Vernalization

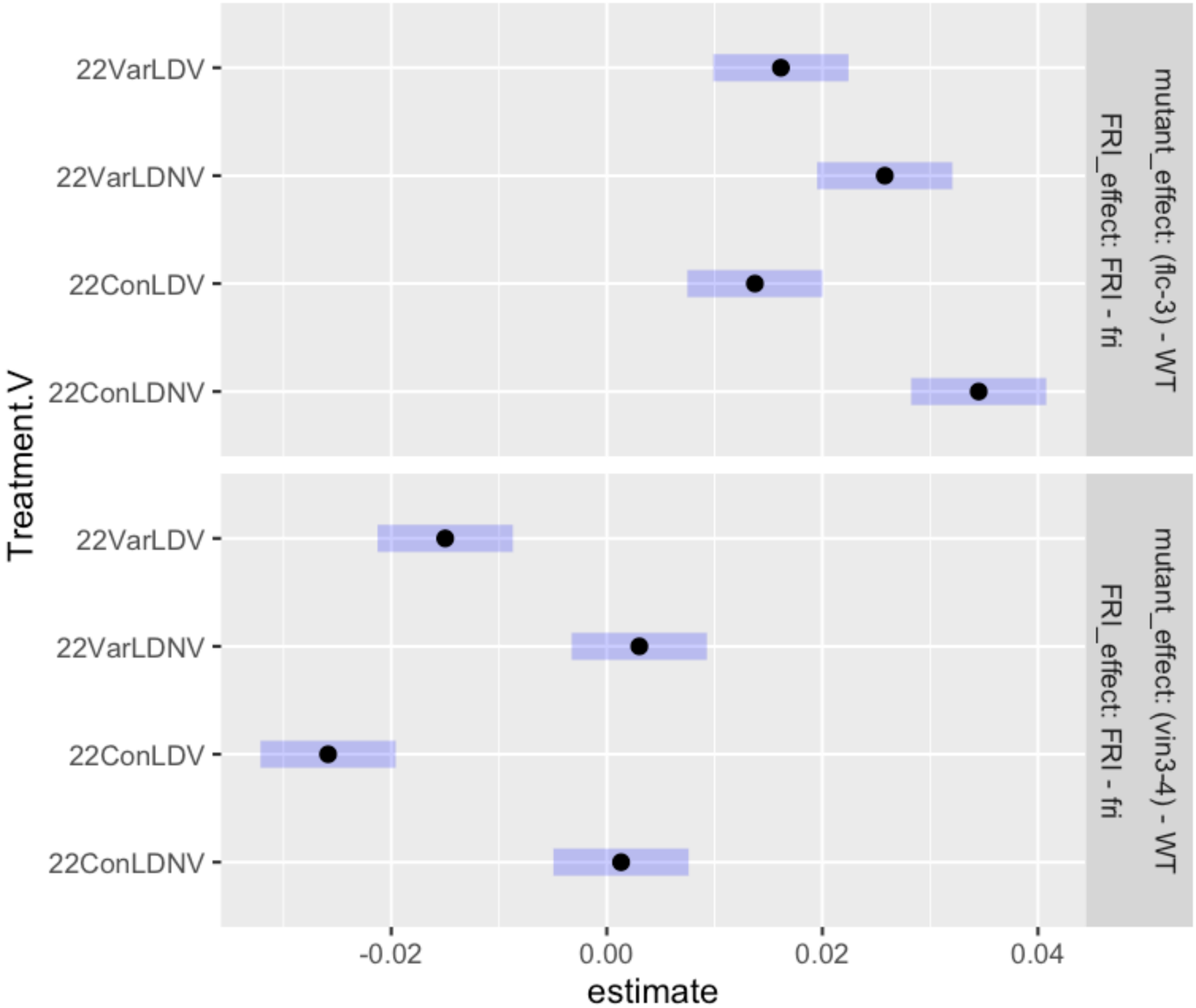


22C + Constant Temp + Vernalization



What changed?

Analysis 2 - FRI effects in different conditions



Summary - Part 1

Start by identifying the **focal treatment effect**

There is never ONE focal treatment effect

Our goal is to report as much about this effect as possible

Magnitude?

Direction?

Scale? Additive? Multiplicative? Inverse/rate? Probability?

Consistency?

Other factors that change its effect

In R:

Load, check data

Fit a model

Construct estimates of effects (contrasts)

Make plots

Part 2 - Experimental Designs

1. Data management
2. Statistics

    Internal vs External validity

    Confidence / uncertainty

    Experimental Units, replication, interspersion

    Blocking

    R tools

Best practices for managing data in Excel

References: Data Organization in Spreadsheets. Broman and Woo 2017

	A	B	C	D	E
1	Plot: 2				
2	Data collecte	Species	Sex	Weight	plate-well
3	1/8/14	NA			1-A01
4	1/8/14	DM	M	44	1-A02
5	1/8/14	DM	M	38	1-A03
6	1/8/14	OL			1-B01
7	1/8/14	PE	M	22	1-B02
8	1/8/14	DM	M	38	1-B03
9	1/8/14	DM	M	48	1-C01
10	1/8/14	DM	M	43	1-C02
11	1/8/14	DM	F	35	1-C03
12	1/8/14	DM	M	43	1-D01
13	1/8/14	DM	F	37	1-D02
14	1/8/14	PF	F	7	1-D03
15	1/8/14	DM	M	45	2-A01
16	1/8/14	OT			2-A02
17	1/8/14	DS	M	157	2-A03
18	1/8/14	OX			2-B01
19					
20	Plot: 3				
21	2/8/14	NA	M	218	2-B02
22	2/8/14	PF	F	7	2-B03
23	2/8/14	DM	M	52	2-C01
24					
25		MEASUREMENT DEVICE NOT CALIBRATED			

What could be improved about this spreadsheet?

Best practices for managing data in Excel

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	A	B	C	D	E
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13	2014-01-08	DM	F	37	1-D02
14	2014-01-08	PF	F	7	1-D03
15	2014-01-08	DM	M	45	2-A01
16	2014-01-08	OT			2-A02
17	2014-01-08	DS	M	157	2-A03
18	2014-01-08	OX			2-B01
19					
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21	2014-02-08	NA	M	218	2-B02
22	2014-02-08	PF	F	7	2-B03
23	2014-02-08	DM	M	52	2-C01
24					
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1. Write dates as YYYY-MM-DD
2. Include a metadata sheet

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6	2014-01-08	OL			1-B01
7	2014-01-08	PE	M	22	1-B02
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13	2014-01-08	DM	F	37	1-D02
14	2014-01-08	PF	F	7	1-D03
15	2014-01-08	DM	M	45	2-A01
16	2014-01-08	OT			2-A02
17	2014-01-08	DS	M	157	2-A03
18	2014-01-08	OX			2-B01
19					
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21	2014-02-08	NA	M	218	2-B02
22	2014-02-08	PF	F	7	2-B03
23	2014-02-08	DM	M	52	2-C01
24					
25		MEASUREMENT DEVICE NOT CALIBRATED			

1. Write dates as YYYY-MM-DD

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	A	B	C
1	**Code**	**Species**	
2	NA	Nabaluia angustifolia	
3	DM	Dialium madagascariense	
4	OL	Oenothera laciniata	
5	PE	Pandanus eydouxia	
6	PF	Pandanus fanningensis	
7	OT	Odontoglossum tenuifolium	
8	DS	Decaspermum salomonense	
9	OX	Oenotrichia maxima	
10			
11	**Weight**	Dry biomass in g	
12	**Plate-well**	plate identifier and well ID	

Sheet1 | Sheet2 | metadata

3. Avoid empty cells

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5	2014-01-08	DM	M	38	1-A03
6	2014-01-08	OL	NA	NA	1-B01
7	2014-01-08	PE	M	22	1-B02
8	2014-01-08	DM	M	38	1-B03
9	2014-01-08	DM	M	48	1-C01
10	2014-01-08	DM	M	43	1-C02
11	2014-01-08	DM	F	35	1-C03
12	2014-01-08	DM	M	43	1-D01
13	2014-01-08	DM	F	37	1-D02
14	2014-01-08	PF	F	7	1-D03
15	2014-01-08	DM	M	45	2-A01
16	2014-01-08	OT	NA	NA	2-A02
17	2014-01-08	DS	M	157	2-A03
18	2014-01-08	OX	NA	NA	2-B01
19	Plot: 3				
20	2014-02-08	NA	M	218	2-B02
21	2014-02-08	PF	F	7	2-B03
22	2014-02-08	DM	M	52	2-C01
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9	2014-01-08	DM	M	48	1	C01
10	2014-01-08	DM	M	43	1	C02
11	2014-01-08	DM	F	35	1	C03
12	2014-01-08	DM	M	43	1	D01
13	2014-01-08	DM	F	37	1	D02
14	2014-01-08	PF	F	7	1	D03
15	2014-01-08	DM	M	45	2	A01
16	2014-01-08	OT	NA	NA	2	A02
17	2014-01-08	DS	M	157	2	A03
18	2014-01-08	OX	NA	NA	2	B01
19	Plot: 3					
20	2014-02-08	NA	M	218	2	B02
21	2014-02-08	PF	F	7	2	B03
22	2014-02-08	DM	M	52	2	C01

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	A	B	C	D	E	F	G
1	Plot	Data collecte	Species	Sex	Weight	plate	well
2	2	2014-01-08	NA	NA	NA	1	A01
3	2	2014-01-08	DM	M	44	1	A02
4	2	2014-01-08	DM	M	38	1	A03
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12	2	2014-01-08	DM	F	37	1	D02
13	2	2014-01-08	PF	F	7	1	D03
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16	2	2014-01-08	DS	M	157	2	A03
17	2	2014-01-08	OX	NA	NA	2	B01
18	3	2014-02-08	NA	M	218	2	B02
19	3	2014-02-08	PF	F	7	2	B03
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21			MEASUREMENT DEVICE NOT CALIBRATED				

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Best practices for managing data in Excel

References: Data Organization in Spreadsheets. Broman and Woo 2017

	A	B	C	D	E	F	G	H
1	Plot	Data collecte	Species	Sex	Weight	plate	well	Calibrated
2	2	2014-01-08	NA	NA	NA	1	A01	Y
3	2	2014-01-08	DM	M	44	1	A02	Y
4	2	2014-01-08	DM	M	38	1	A03	Y
5	2	2014-01-08	OL	NA	NA	1	B01	Y
6	2	2014-01-08	PE	M	22	1	B02	Y
7	2	2014-01-08	DM	M	38	1	B03	Y
8	2	2014-01-08	DM	M	48	1	C01	Y
9	2	2014-01-08	DM	M	43	1	C02	Y
10	2	2014-01-08	DM	F	35	1	C03	Y
11	2	2014-01-08	DM	M	43	1	D01	Y
12	2	2014-01-08	DM	F	37	1	D02	Y
13	2	2014-01-08	PF	F	7	1	D03	Y
14	2	2014-01-08	DM	M	45	2	A01	Y
15	2	2014-01-08	OT	NA	NA	2	A02	Y
16	2	2014-01-08	DS	M	157	2	A03	N
17	2	2014-01-08	OX	NA	NA	2	B01	Y
18	3	2014-02-08	NA	M	218	2	B02	N
19	3	2014-02-08	PF	F	7	2	B03	Y
20	3	2014-02-08	DM	M	52	2	C01	Y

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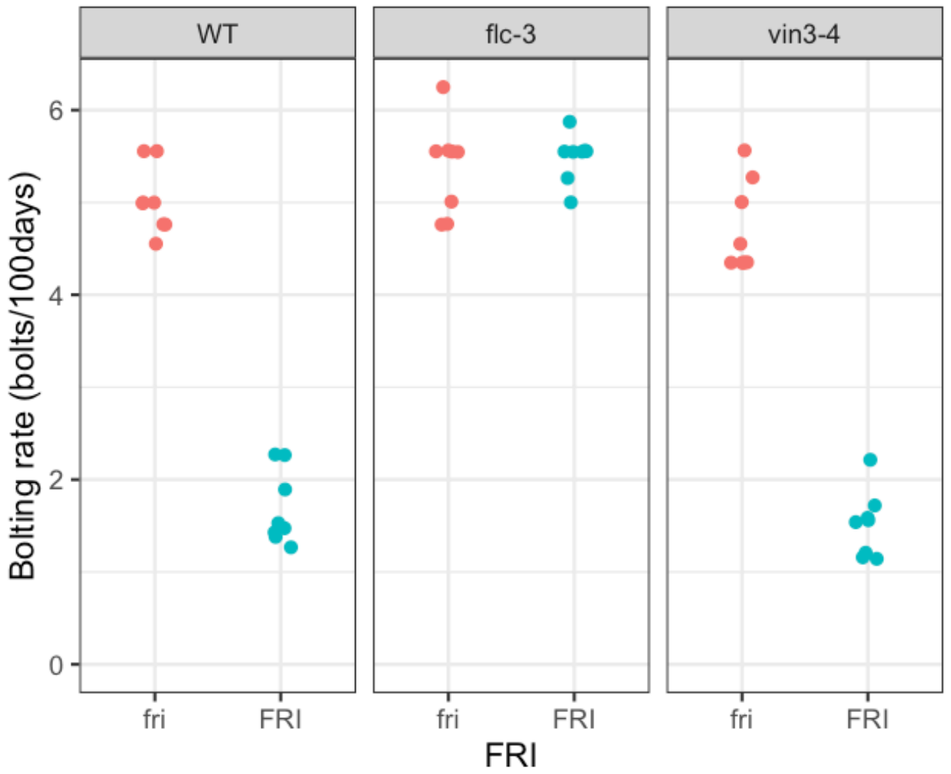
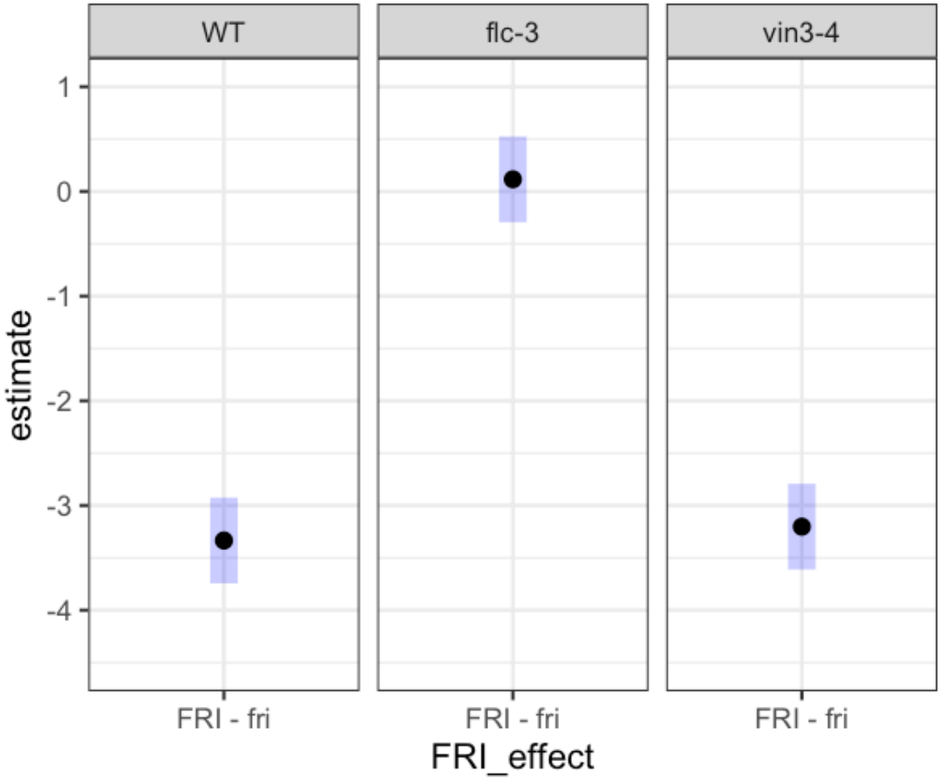
3. Avoid empty cells
4. Put only 1 thing in each cell
5. Make it a rectangle
6. Don’t use font or highlighting as data
7. Use Data Validation to help data entry
8. Export as .csv for analysis

Statistics

Data



Conclusions



Are these conclusions **valid**?

What do the confidence intervals mean?

How can we make confidence intervals shorter?

Do we **always** want to?

Validity of conclusions

Are our estimates as good as they could be?

Are we accuractly communicating the confidence we have in our conclusions?

Internal Validity

Statements about the results of *this experiment*

"past validity" - use past tense

External Validity

Extrapolations to broader conditions

"future validity" - use present/future tense

Validity requires the correct pairing of Experimental Design, Analysis methods, and Conclusion statements

The same experiment can be valid or invalid depending on the analysis

The same analysis can be valid or invalid depending on the Conclusion statements

The same experiment can be validly analyzed in different ways depending on the scope

Experimental Design

Optimizing experimental strategies to get the most out of your work

Maximize “Gain in Knowledge” per $$, time

Components:

- Response
- Treatments
- Design
- Analysis

Tools:

- Experimental Units
- Blocks / Replication
- Design Tables

Experimental Units

Unit of replication of a specific level of a treatment

Fundamental building block of any experiment

The **smallest** unit of experimental material to which a **single treatment** (or treatment combination) is assigned by the experimenter and which is dealt with **independently** of other such systems **under that treatment** at **all stages in the experiment** at which important variation may enter.

Kozlov and Hurlbert 2006

Each experimental unit get its treatment **independently**

Each experimental unit is **equally likely** to be assigned each treatment

Experimental units shouldn't **interfere** with each other

Experimental units should be **randomly** selected from a **reference population**

Experimental units of **different treatments** should be **interspersed** both temporally and spatially

Example 1

40 pots are planted with pepper plants

1 plant per pot

2 hot and 2 cold growth chambers

10 plants per chamber

2 leaves harvested per plant (pot)

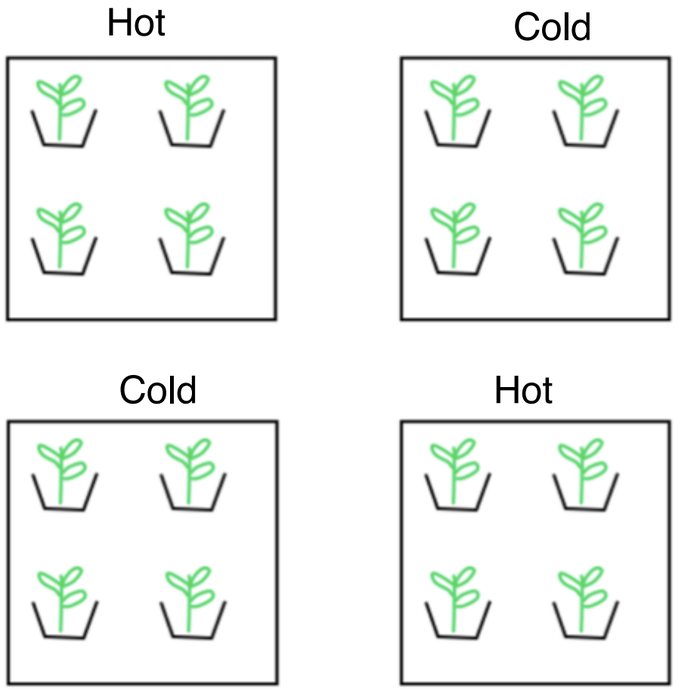
RNA extracted from each leaf

expression of the gene *sp1* measured 3 times per RNA sample

What is the **treatment**?

What is the **Experimental Unit**?

Example 1



Replicate of one Temperature:	Chamber
Replicate of one Chamber:	Plant
Replicate of one Plant:	Leaf

Key idea: **Interspersion**

If you can draw a “box” around a group of plants of the same treatment

and *accidental* variation can affect all plants in that group

Then the individual plant is **not the Experimental Unit**

Randomization can create interspersion

But not always. Interspersion is always important

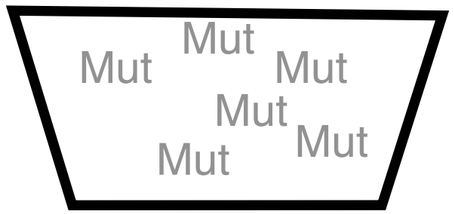
Example 2

To study the effect of mutating the MC1R gene on fish fin colors, a researcher spends 2 years generating a knock-out mutant.

She places 6 fish of the wild-type strain in one tank and 6 fish of the mutant strain in a second tank.

When they get to 5cm in length, she measures the fin color of each fish

What is the experimental unit for **the effect of MC1R** on fin coloration?



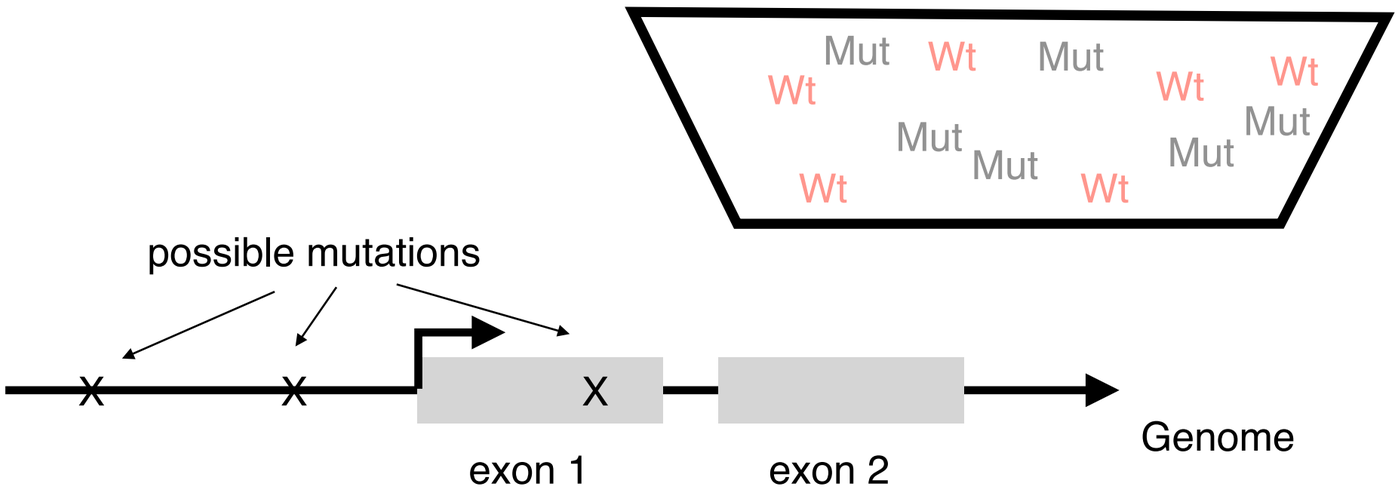
Example 2

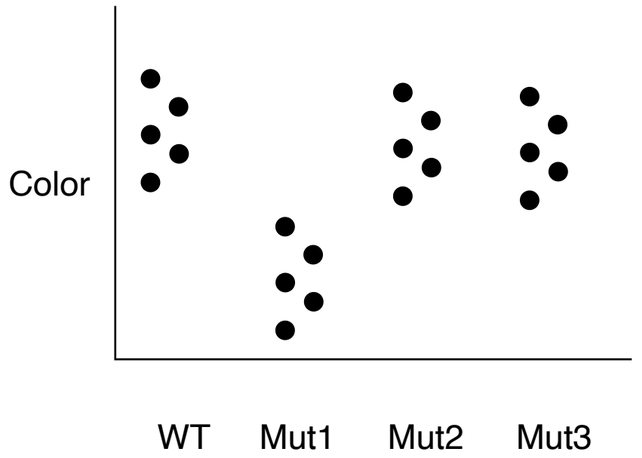
To study the effect of mutating the MC1R gene on fish fin colors, a researcher spends 2 years generating a knock-out mutant.

She places 6 fish of the wild-type strain in one tank and 6 fish of the mutant strain in a second tank.

When they get to 5cm in length, she measures the fin color of each fish

She **TAGS 6 FISH** of the wild-type strain and 6 of the mutant strain and grows them **All IN ONE TANK**





Is it the **Gene**?

Or just the specific mutation?

Experimental Units - Key Ideas

Every experiment needs experimental units

Valid measurements of treatment effects

Each experimental unit is specific to **one treatment level**

Each experimental unit is dealt with independently of every other experimental unit **throughout the whole experiment**

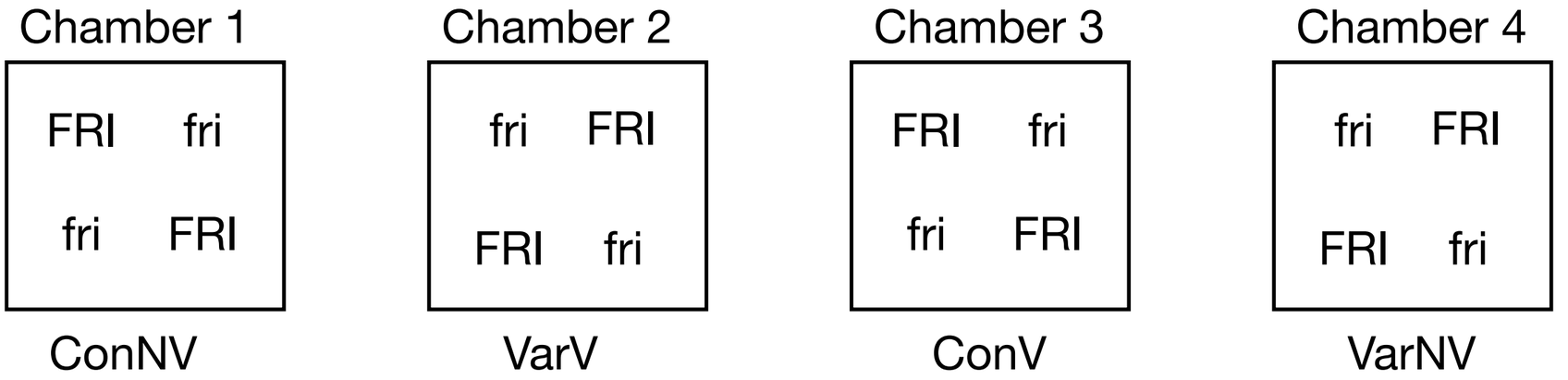
Experimental units of different treatment levels are **interspersed** in **all dimensions that important variation can enter**

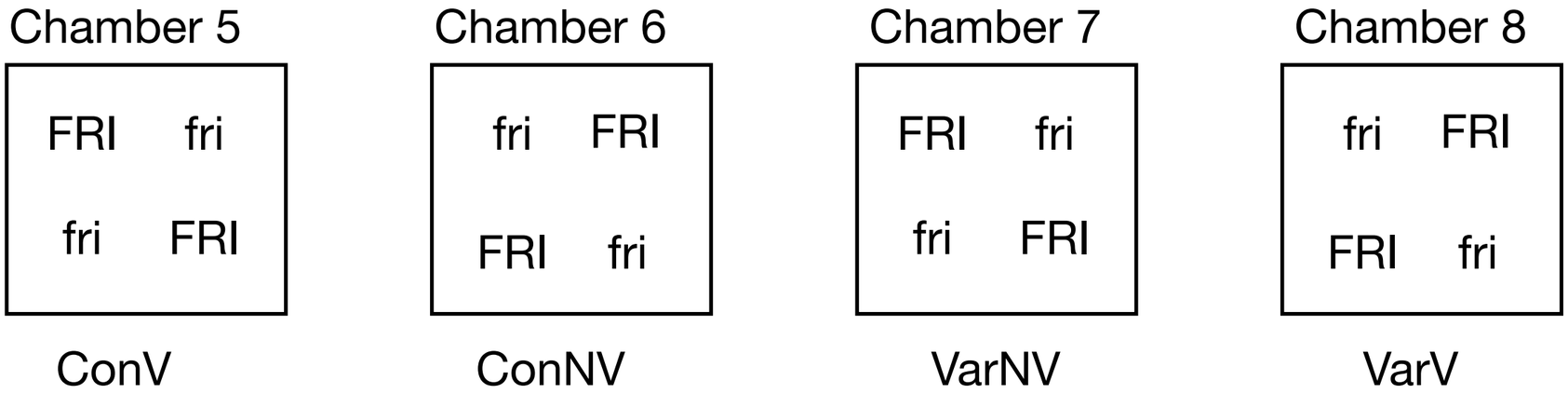
Draw out the experimental layout

Can you draw a “box” around multiple units of the same treatment level?

Each **treatment factor** can have a **different Experimental Unit factor**

Burghardt *et al* 2016



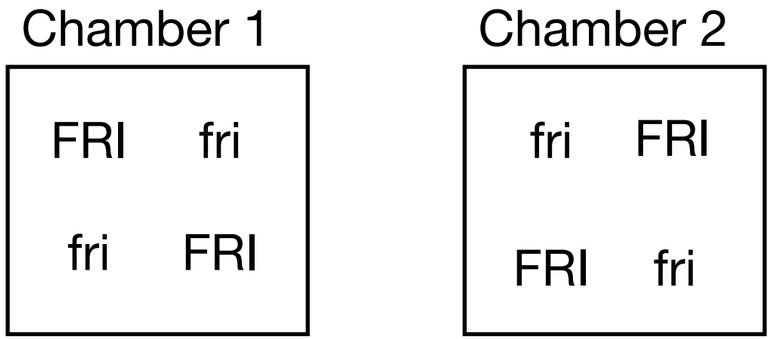


What is the Experimental Unit for the **Environment** treatment? Chamber

What is the Experimental Unit for the **FRI** treatment? Pot

Blocks

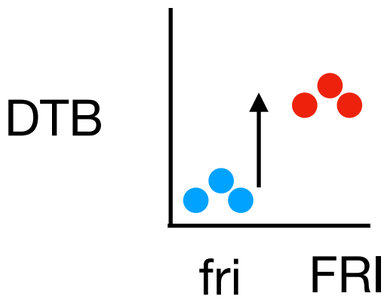
Block = mini experiment within a bigger experiment

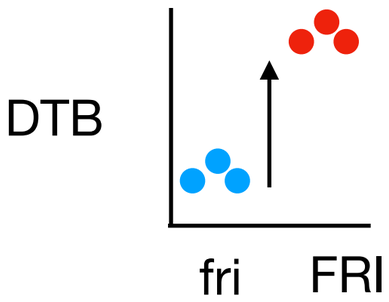


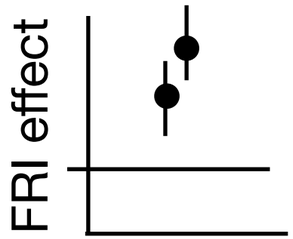
Each chamber has a **complete experiment**

1+ experimental units for 2+ treatment levels

We can measure the **treatment effect** within each chamber







Replicates of treatment **effects**

Necessary for **external validity**

Sometimes necessary / useful for interspersion (internal validity)

Why use blocks?

Chamber 1

FRI	fri
fri	FRI

Chamber 2

fri	FRI
FRI	fri

vs

Chamber 1

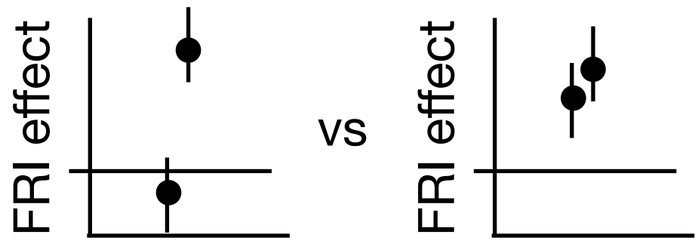
FRI	fri	fri	FRI
fri	FRI	FRI	fri

1. Experimental Precision Internal Validity

Can't fit enough plants in one chamber without interferring with each other

Bigger chambers have less precise environmental control

FRI effects will be more consistent in smaller chambers



2. Generalizability External Validity

We need to see how much the FRI effect varies to extrapolate conclusions to new conditions

Chambers always differ *somewhat* from each other, so FRI effects will too

How to block

1. Repeat the whole experiment

   “Best” replication Necessary for treatments that cannot be interspersed

2. Identify groups of experimental units that you expect to be more homogeneous

   Plants/pots within a chamber

   Assay plate

   Field location

   Field location

   Undergrad technician

3. Give this group of EU a **unique name**, record in your data table

4. Randomize treatment levels to EU **within each block**

5. (Optional) Run experiment for each block separately

   Measure treatment effects for each block separately

Blocks - Summary

Blocks are mini-experiments

- Increase precision within a specific condition
- Help measure variation in treatment effects among conditions

Most experiments use blocks!

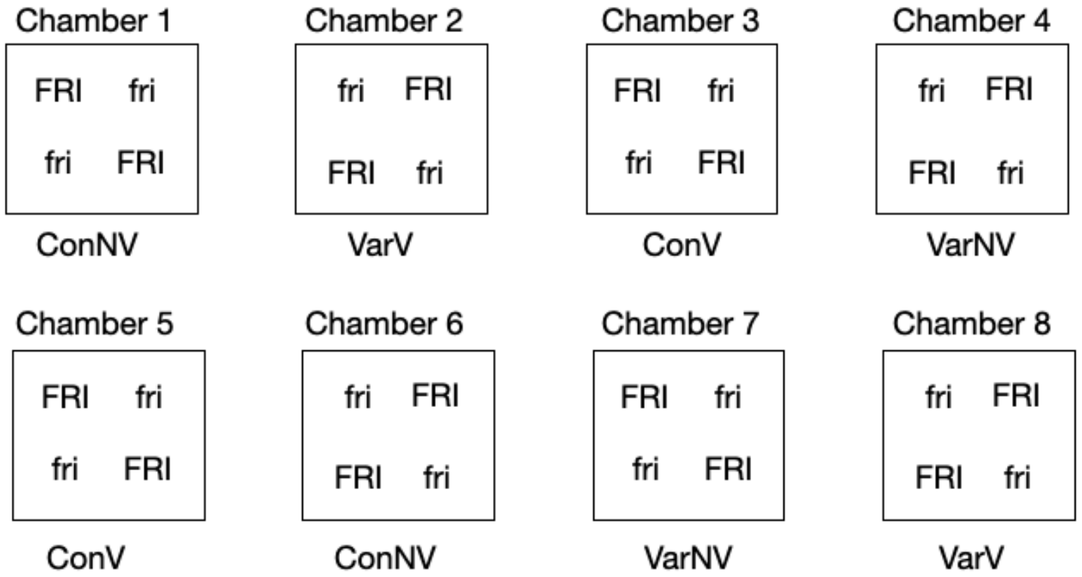
- Any non-treatment factor containing 2+ treatment levels is a block
- Can have multiple blocking factors
  - Usually best to overlay blocks

Chamber 1	Chamber 2	Chamber 3
↓	↓	↓
Undergrad 1	Undergrad 2	Undergrad 3
↓	↓	↓
Plate 1	Plate 2	Plate 3

- “Confound” the effects of each blocking factor
  - Our goal isn’t to characterize the blocks themselves

Analysis of experiments

How do you communicate your experimental design and analysis goals to R?



Treatment	**Experimental Unit**	**Block(s)**
Treatment.V	Chamber	None
FRI	Pot	Chamber

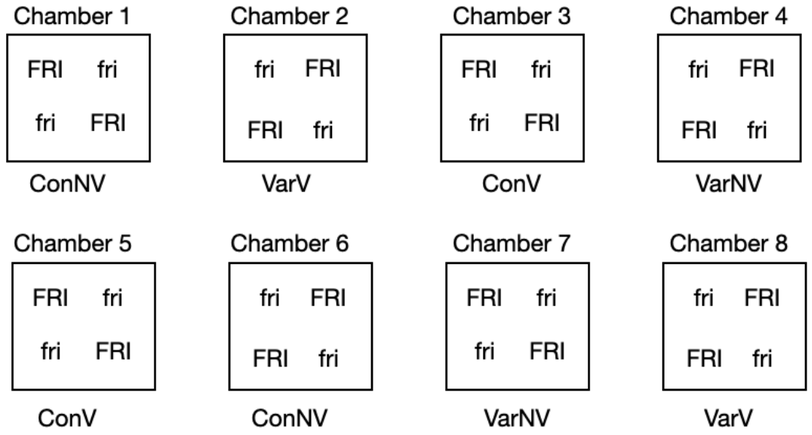
Goal:

Effect of Treatment.V on FRI effect on flowering time

What R sees

	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
2	7	Col	fri	WT	22ConLDNV	1	20
3	8	Col	fri	WT	22ConLDNV	1	21
4	9	Col FRI	FRI	WT	22ConLDNV	1	53

Design Table -> Model statement



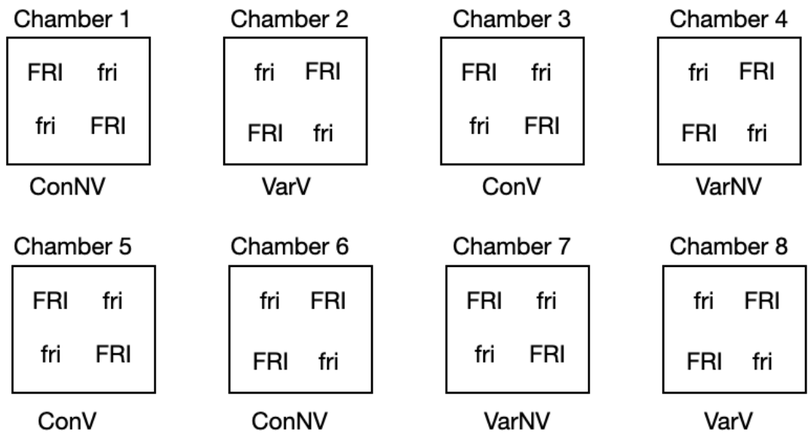
	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
2	7	Col	fri	WT	22ConLDNV	1	20
3	8	Col	fri	WT	22ConLDNV	1	21
4	9	Col FRI	FRI	WT	22ConLDNV	1	53

Structure	Variable	# levels	Block	EU
Response	Days.to.Bolt	64		
focal treatment	FRI	2	Chamber	Pot
moderator treatment	Treatment.V	4	None	Chamber
combo treatment	FRI:Treatment.V	8	Chamber	Pot
Design	Chamber	8		
	Pot	64		
	FRI:Chamber	16		
	FRI:Treatment.V:Chamber	64		

Response Treatments Design

model = lmer(Days.to.Bolt ~ FRI + Treatment.V + FRI:Treatment.V + (1|Chamber) + (1|FRI:Chamber))

Design Table - 1. Response



Structure	Variable	# levels	Block	EU
Response	Days.to.Bolt	64		

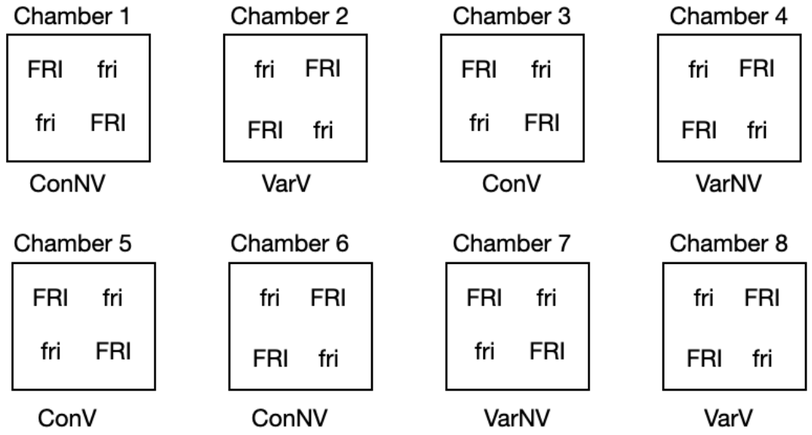
Variable: name of column in data.frame

or inverse(Days.to.Bolt/100)

 # levels: # rows in data.frame

	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
2	7	Col	fri	WT	22ConLDNV	1	20
3	8	Col	fri	WT	22ConLDNV	1	21
4	9	Col FRI	FRI	WT	22ConLDNV	1	53

Design Table - 2. Treatment



Structure	Variable	# levels	Block	EU
Response	Days.to.Bolt	64		
focal treatment	FRI	2	Chamber	Pot
moderator treatment	Treatment.V	4	None	Chamber

	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
2	7	Col	fri	WT	22ConLDNV	1	20
3	8	Col	fri	WT	22ConLDNV	1	21
4	9	Col FRI	FRI	WT	22ConLDNV	1	53

Variable: name of column in data.frame

 # levels: # levels of each treatment

Block and **EU:**

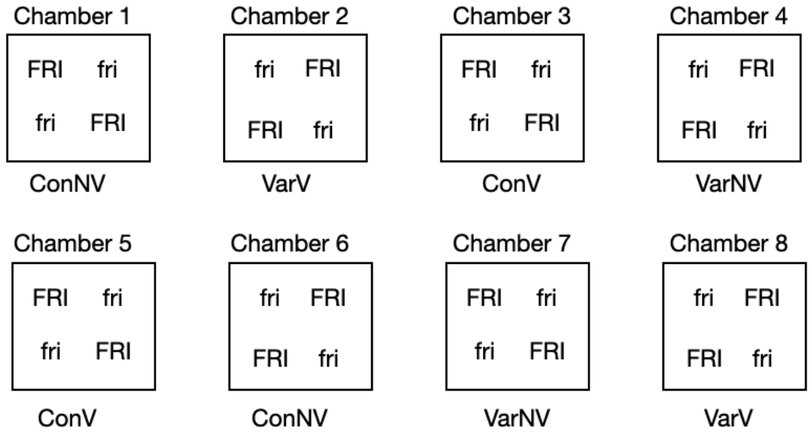
Based on the design

Use the column names in data.frame

focal and moderator treatment

When 2+ treatments, declare 1 “focal”

Design Table - 2. Treatment combos



Structure	Variable	# levels	Block	EU
Response	Days.to.Bolt	64		
focal treatment	FRI	2	Chamber	Pot
moderator treatment	Treatment.V	4	None	Chamber
combo treatment	FRI:Treatment.V	8	Chamber	Pot

	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
2	7	Col	fri	WT	22ConLDNV	1	20
3	8	Col	fri	WT	22ConLDNV	1	21
4	9	Col FRI	FRI	WT	22ConLDNV	1	53

Combos are combined variables

combine names with "**:**" *e.g.* FRI:Treatment.V

	ConNV	VarNV	ConV	VarV
fri	fri:ConNV	fri:VarNV	fri:ConV	fri:VarV
FRI	FRI:ConNV	FRI:VarNV	FRI:ConV	FRI:VarV

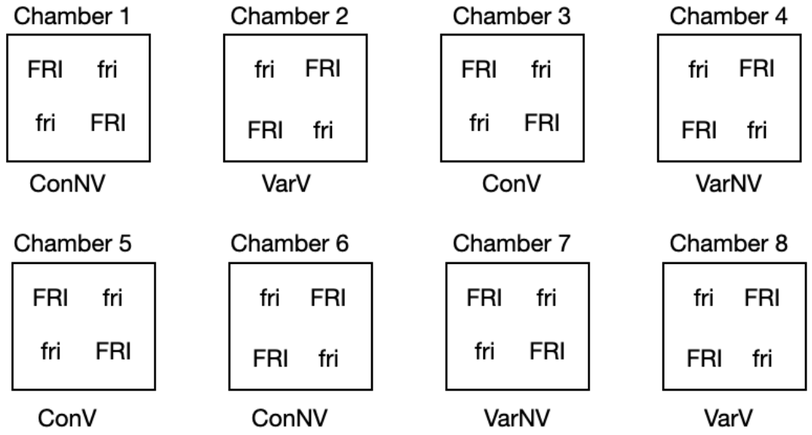
Terminology:

"FRI" and "Treatment.V" are **crossed**

rows and columns have 2+ entries

 # levels: # unique combinations *in the experiment*

Design Table - 3. Design



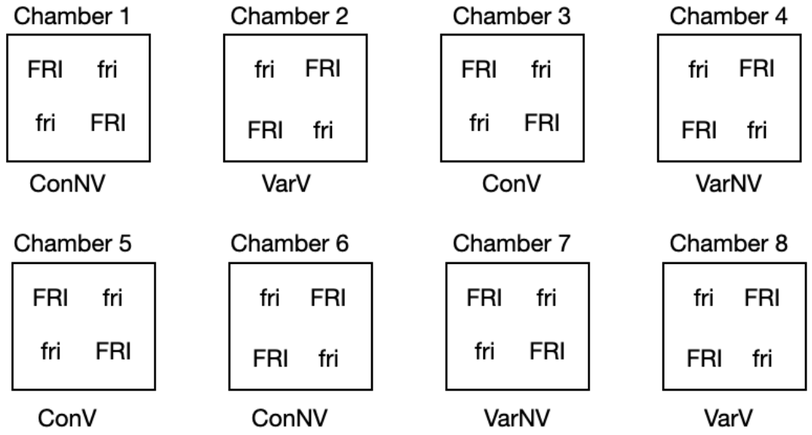
	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
2	7	Col	fri	WT	22ConLDNV	1	20
3	8	Col	fri	WT	22ConLDNV	1	21
4	9	Col FRI	FRI	WT	22ConLDNV	1	53

Structure	Variable	# levels	Block	EU
Response	Days.to.Bolt	64		
focal treatment	FRI	2	Chamber	Pot
moderator treatment	Treatment.V	4	None	Chamber
combo treatment	FRI:Treatment.V	8	Chamber	Pot
Design	Chamber	8		
	Pot	64		

Variable:

List all Block and EUs Check that they are named **uniquely**!

Design Table - 3. Design



	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
2	7	Col	fri	WT	22ConLDNV	1	20
3	8	Col	fri	WT	22ConLDNV	1	21
4	9	Col FRI	FRI	WT	22ConLDNV	1	53

Structure	Variable	# levels	Block	EU
Response	Days.to.Bolt	64		
focal treatment	FRI	2	Chamber	Pot
moderator treatment	Treatment.V	4	None	Chamber
combo treatment	FRI:Treatment.V	8	Chamber	Pot
Design	Chamber	8		
	Pot	64		
	FRI:Chamber	16		
	FRI:Treatment.V:Chamber	64		

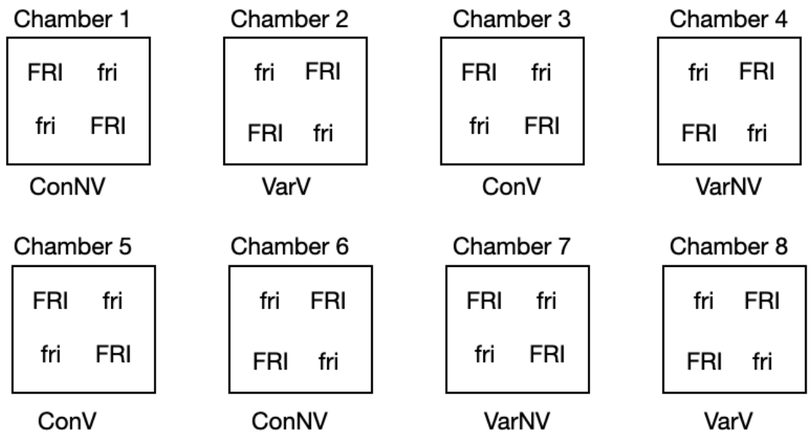
Variable:

List all Block and EUs Check that they are named **uniquely**!

Form all possible **combination terms** among **crossed variables** count # levels

all Treatment:Block some Block:Block

Crossed vs Nested vs Aliased



	FRI	Treatment.V	Chamber	Pot
# levels	2	4	8	64

	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
2	7	Col	fri	WT	22ConLDNV	1	20
3	8	Col	fri	WT	22ConLDNV	1	21
4	9	Col FRI	FRI	WT	22ConLDNV	1	53

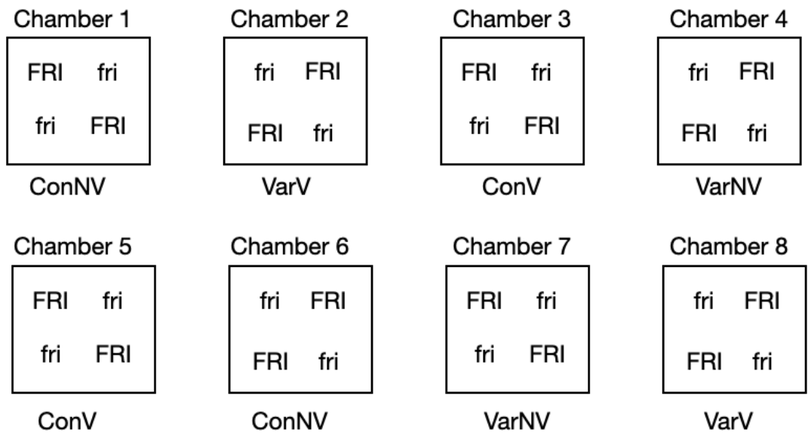
Treatment.V

FRI	ConNV	VarNV	ConV	VarV
fri	✓	✓	✓	✓
FRI	✓	✓	✓	✓

Crossed

Crossed Rows and Columns have 2+ entries

Crossed vs Nested vs Aliased



	FRI	Treatment.V	Chamber	Pot
# levels	2	4	8	64

	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
2	7	Col	fri	WT	22ConLDNV	1	20
3	8	Col	fri	WT	22ConLDNV	1	21
4	9	Col FRI	FRI	WT	22ConLDNV	1	53

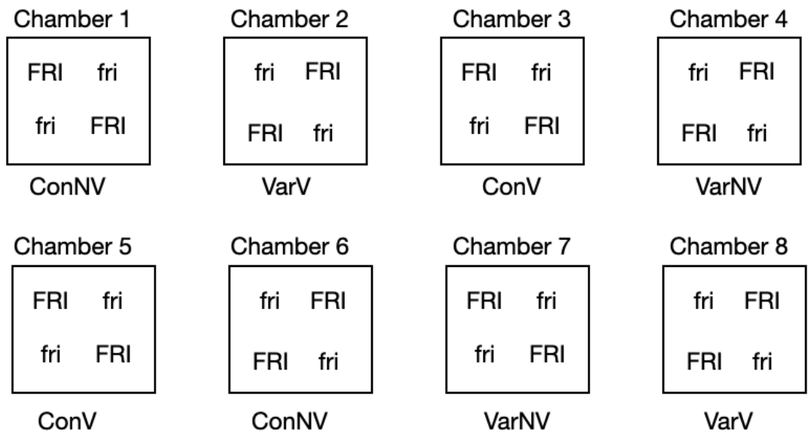
Chamber

FRI	Cham1	Cham2	Cham3	Cham4	Cham5	Cham6
fri	✓	✓	✓	✓	✓	✓
FRI	✓	✓	✓	✓	✓	✓

Crossed

Crossed Rows and Columns have 2+ entries

Crossed vs Nested vs Aliased

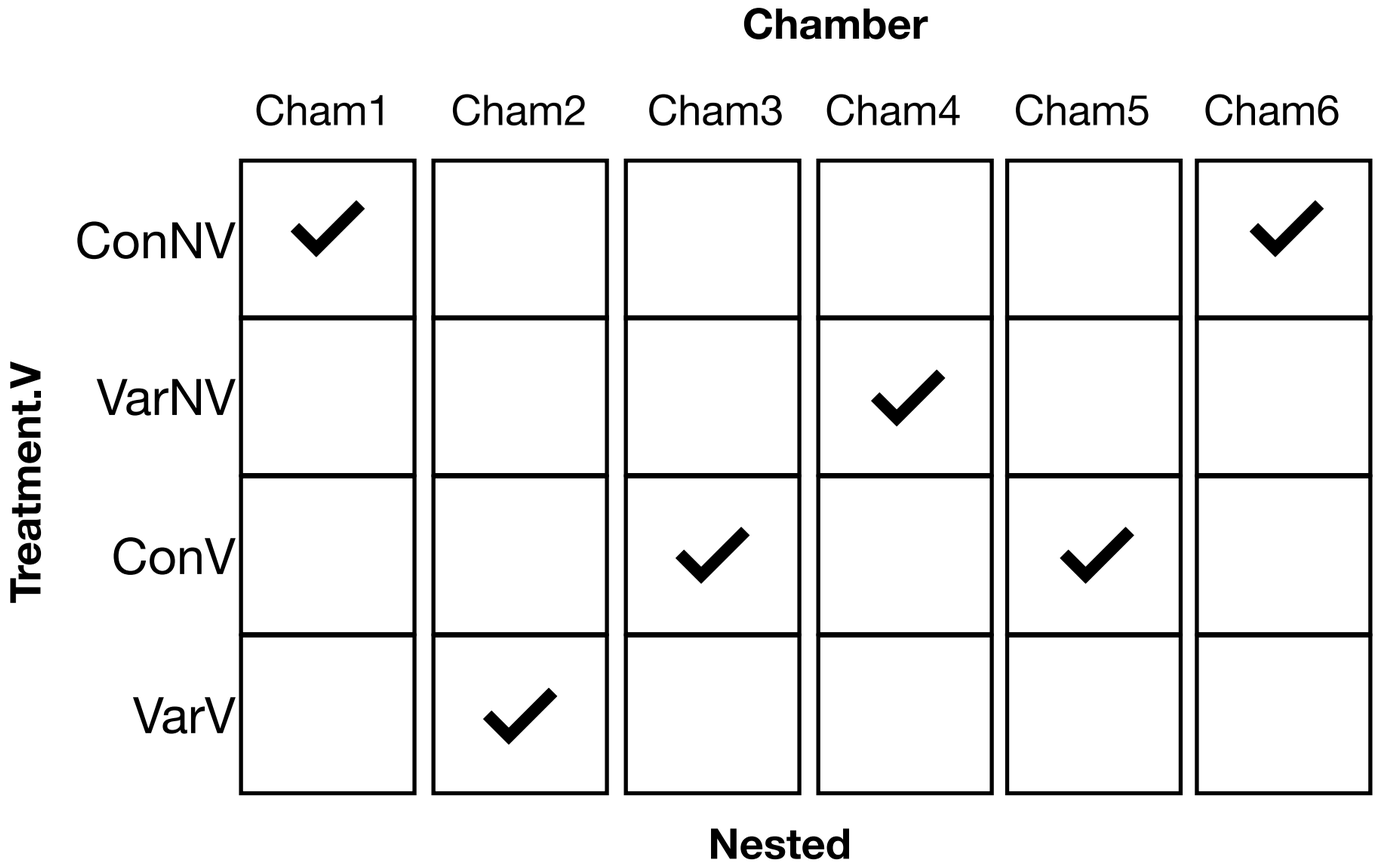


	FRI	Treatment.V	Chamber	Pot
# levels	2	4	8	64

	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
2	7	Col	fri	WT	22ConLDNV	1	20
3	8	Col	fri	WT	22ConLDNV	1	21
4	9	Col FRI	FRI	WT	22ConLDNV	1	53

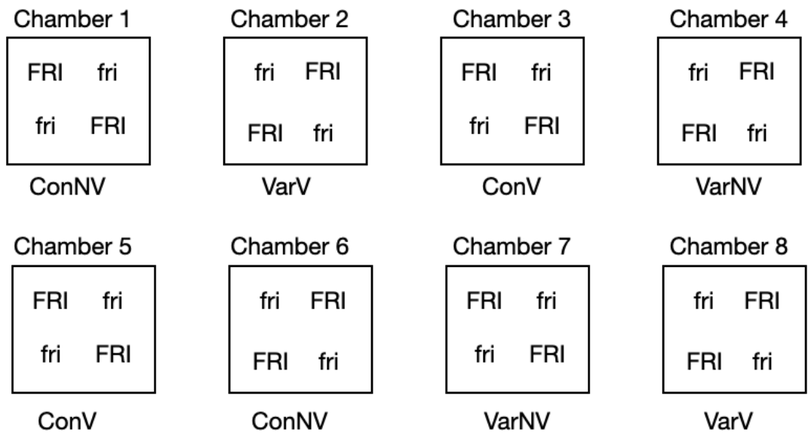
Crossed Rows and Columns have 2+ entries

Nested Rows **or** Columns have 2+ entries. The other has only 1



Rows **or** Columns have 2+ entries. The other has only 1

Crossed vs Nested vs Aliased



	FRI	Treatment.V	Chamber	Pot
# levels	2	4	8	64

(Treatment.V:Chamber):Chamber

ConNV:Cham1 —— Cham1

VarV:Cham2 —— Cham2

ConV:Cham3 —— Cham3

~~ConV:Cham4~~ Cham4

~~ConV:Cham5~~

Aliased

one-to-one labels

	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
2	7	Col	fri	WT	22ConLDNV	1	20
3	8	Col	fri	WT	22ConLDNV	1	21
4	9	Col FRI	FRI	WT	22ConLDNV	1	53

Crossed Rows and Columns have 2+ entries

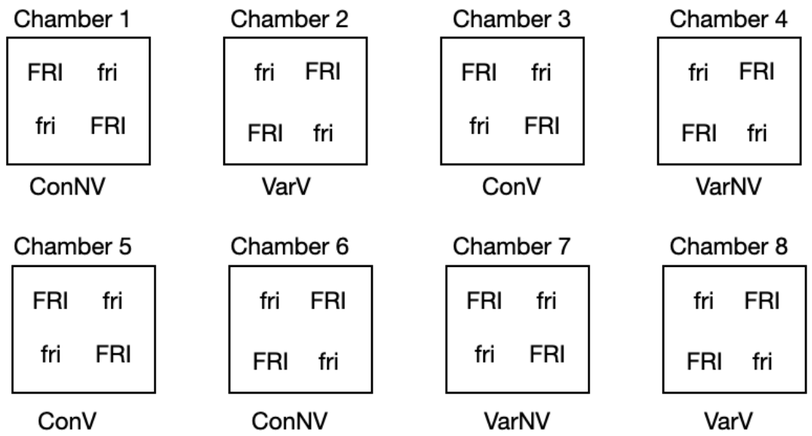
Nested Rows **or** Columns have 2+ entries. The other has only 1

Aliased one-to-one labels

4*8=32 possible levels

only 8 *exist*

Crossed vs Nested vs Aliased



	FRI	Treatment.V	Chamber	Pot
# levels	2	4	8	64

Genotype : FRI

Col FRI —— FRI

Col —— fri **Aliased**

one-to-one labels

Alternate names for the same “thing”

All observations with Genotype == ‘Col FRI’

also have FRI == ‘FRI’

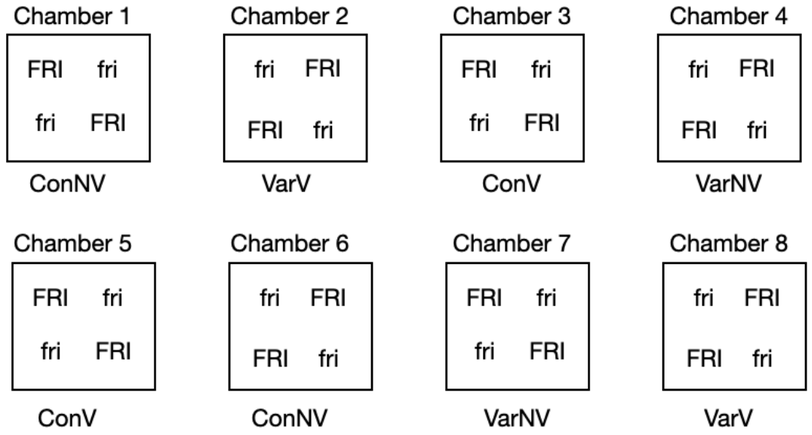
	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
2	7	Col	fri	WT	22ConLDNV	1	20
3	8	Col	fri	WT	22ConLDNV	1	21
4	9	Col FRI	FRI	WT	22ConLDNV	1	53

Crossed Rows and Columns have 2+ entries

Nested Rows **or** Columns have 2+ entries.
The other has only 1

Aliased one-to-one labels

Design Table - 3. Design



	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
2	7	Col	fri	WT	22ConLDNV	1	20
3	8	Col	fri	WT	22ConLDNV	1	21
4	9	Col FRI	FRI	WT	22ConLDNV	1	53

Structure	Variable	# levels	Block	EU
Response	Days.to.Bolt	64		
focal treatment	FRI	2	Chamber	Pot
moderator treatment	Treatment.V	4	None	Chamber
combo treatment	FRI:Treatment.V	8	Chamber	Pot
Design	Chamber	8		
	Pot	64		
	FRI:Chamber	16		
	FRI:Treatment.V:Chamber	64		

Crossed Rows and Columns have 2+ entries

Nested Rows **or** Columns have 2+ entries. The other has only 1

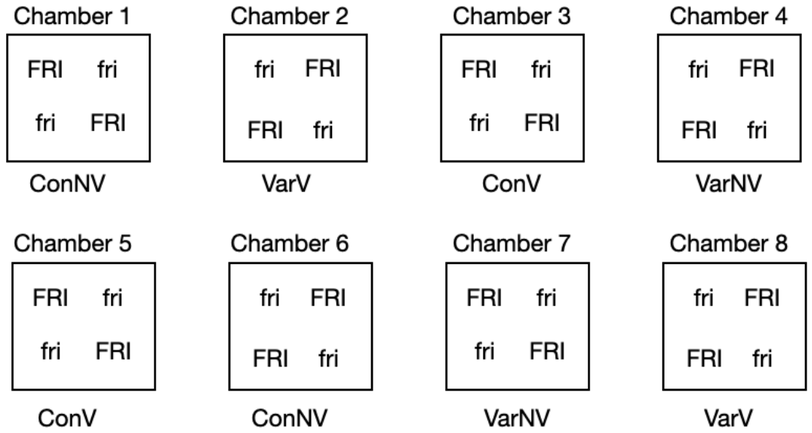
Aliased one-to-one labels

Keep adding rows for any **crossed** combos

If B is **nested in** A, or **aliased with** A, don't form a combo

If C and A are **aliased**, don't need C (unless it is an EU)

Design Table - 4. Model



	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
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Structure	Variable	# levels	Block	EU
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combo treatment	FRI:Treatment.V	8	Chamber	Pot
Design	Chamber	8		
	~~Pot~~	~~64~~		
	FRI:Chamber	16		
	~~FRI:Treatment.V:Chamber~~	~~64~~		

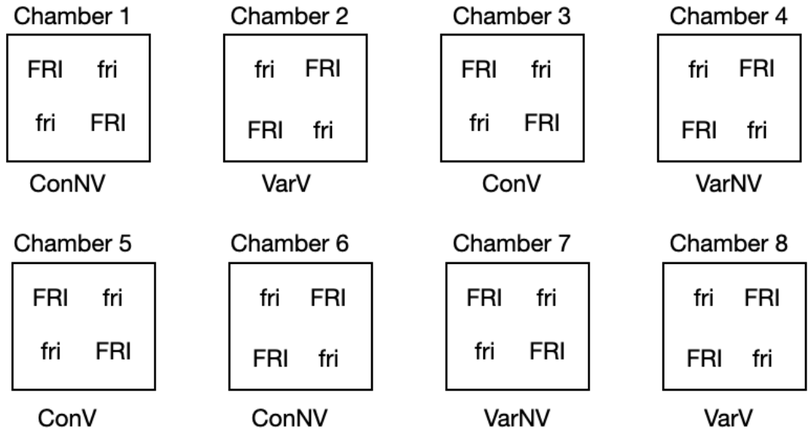
1. Drop rows with same # levels as the Response

2. List all other terms, separated by “+”

Response ~ FRI + Treatment.V + FRI:Treatment.V + Chamber + FRI:Chamber

3. Convert **EUs**, terms **nested in EUs**, and (usually) **Treatment:Block combos** to random

Design Table - 4. Model



	Pot	Genotype	FRI	mutant	Treatment.V	Chamber	Days.to.Bolt
	<dbl>	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	3	Col FRI	FRI	WT	22ConLDNV	1	70
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Structure	Variable	# levels	Block	EU
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Design	Chamber	8		
	~~Pot~~	~~64~~		
	FRI:Chamber	16		
	~~FRI:Treatment.V:Chamber~~	~~64~~		

1. Drop rows with same # levels as the Response

2. List all other terms, separated by “+”

Response ~ FRI + Treatment.V + FRI:Treatment.V + (1| Chamber) + (1|FRI:Chamber)

3. Convert **EUs**, terms **nested in EUs**, and (usually) **Treatment:Block combos** to random (1|Variable)

model function:

Any random terms: lmer()

NO random terms: lm()

Analysis

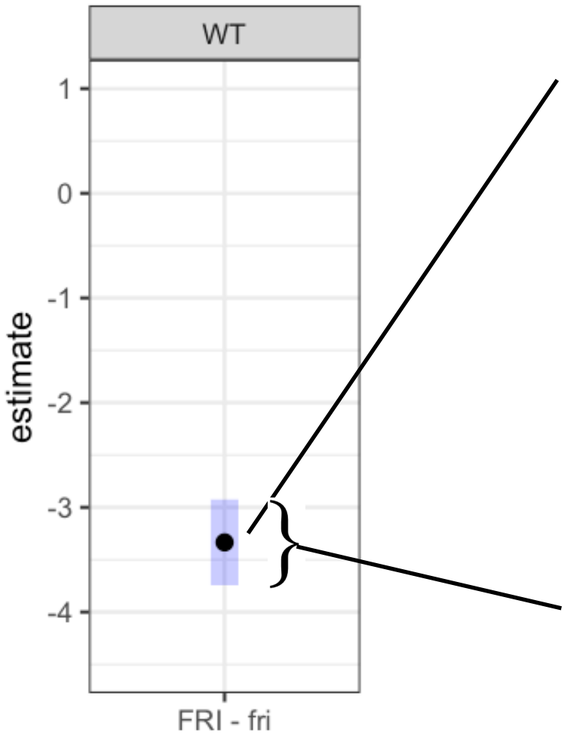
Once you have your data loaded and your model statement written

The analysis is the same as we saw earlier

1. Fit model
2. Calculate means for each treatment
3. Calculate treatment effects
4. If moderator treatments, regroup effects, calculate moderator treatment effects on focal treatment effects
5. Report treatment effect **estimates** and **Confidence Intervals**

Confidence Intervals

Summary of our knowledge after an experiment



Estimate = “measurement” of the thing we’re trying to study

always the average of something

Average effect of adding a functional FRI allele to a WT (Col-0) plant on the **rate of bolting** when grown in the 22-Con-LD-NV condition

Remember: it doesn’t mean that this is **always** the effect!

Confidence Interval = Range of *plausible* errors in our measurement

Using this experimental design

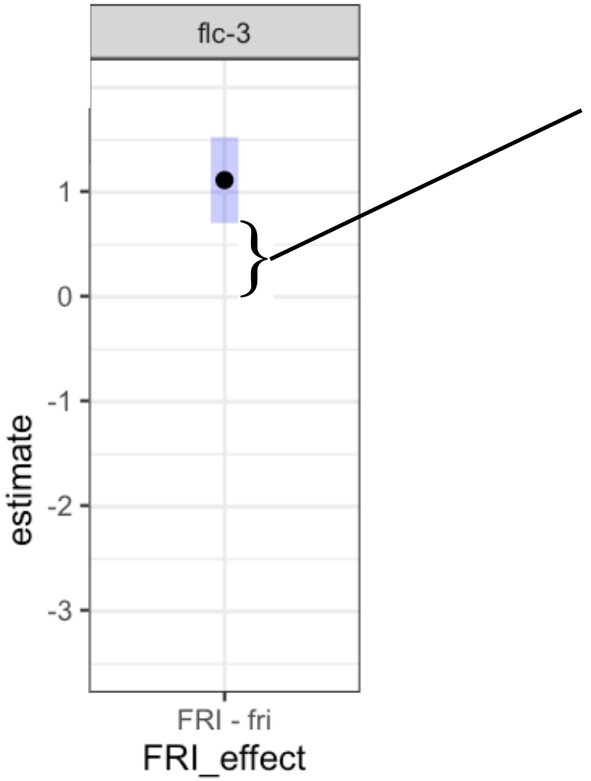
If we were to repeat the experiment many times in exactly the same way

NOT: Range of plausible *treatment effects*

Shorter interval = More knowledge = More confidence in the conclusions

p-values

Evidence that the TRUE value is NOT equal to zero

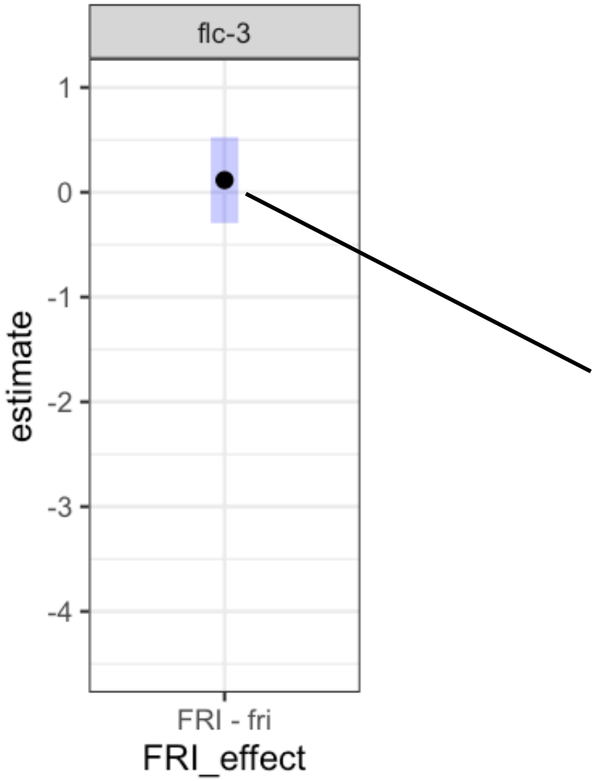


If a 95% Confidence Interval doesn't cross zero, then the p-value < 0.05

Smaller p-values = stronger evidence that the TRUE value is not zero

p-values

Evidence that the TRUE value is NOT equal to zero



If a 95% Confidence Interval doesn't cross zero, then the p-value < 0.05

Smaller p-values = stronger evidence that the TRUE value is not zero

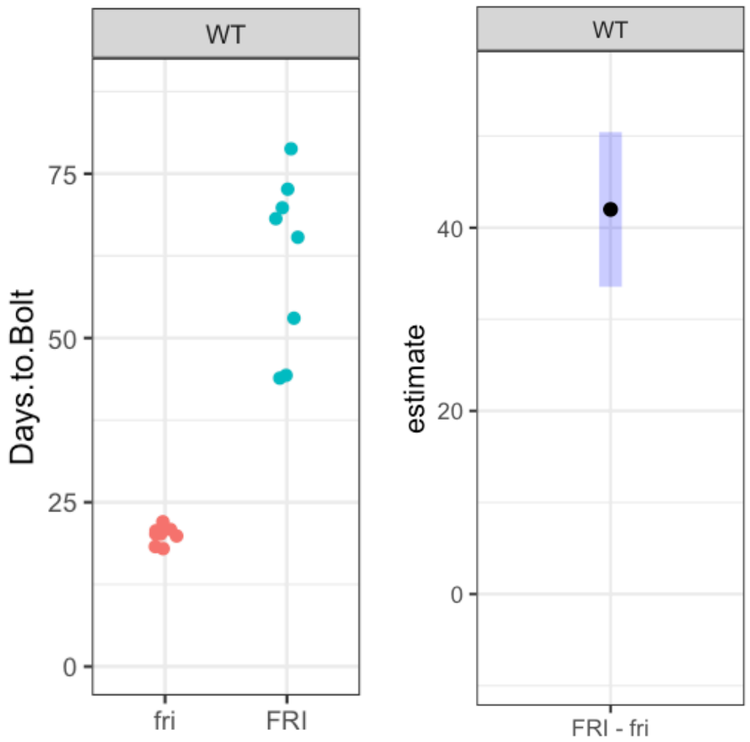
Here, p will be large (~0.7 or so)

Can we conclude that the FRI effect IS zero?

No! There are lots of other plausible values near to zero

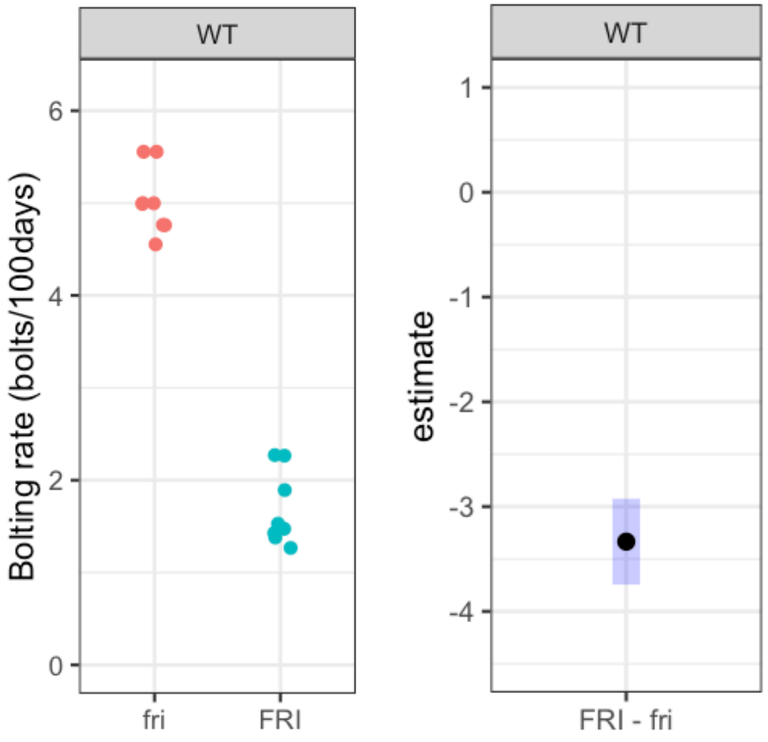
What determines the length of a Confidence Interval?

Days.to.Bolt



Why is this CI larger?

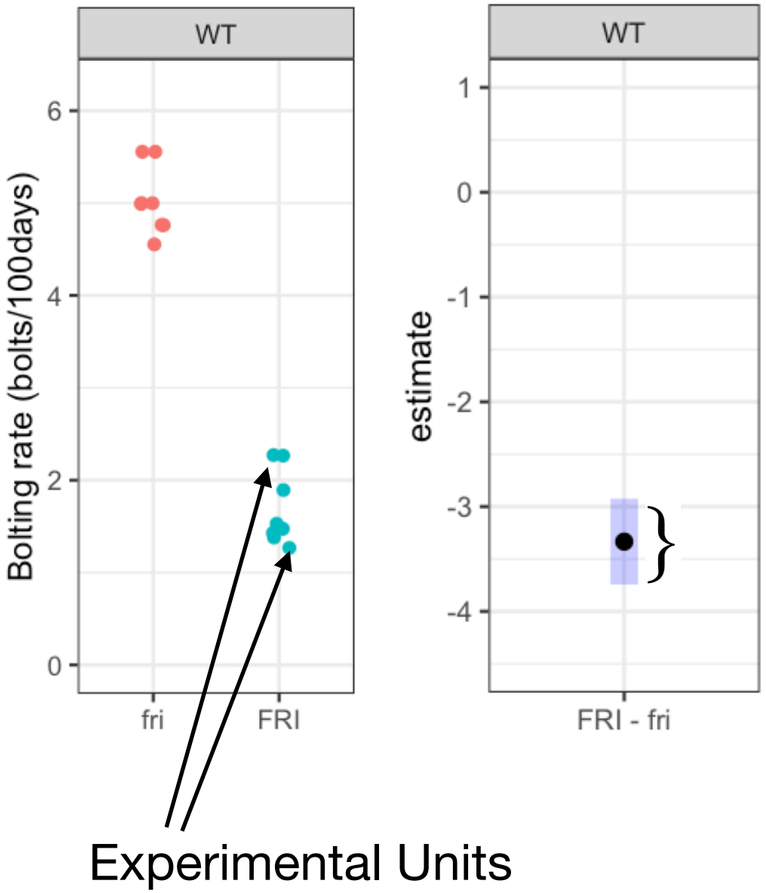
inverse(Days.to.Bolt/100)



We are more confident in the **average effect size** in thiese data

Because the **variation in effect size** *measured in this way* is smaller

What determines the length of a Confidence Interval?



Experimental Units

Not necessarily “replicates”

Variation in effect size

times we measure the effect

Measurement error per measurement

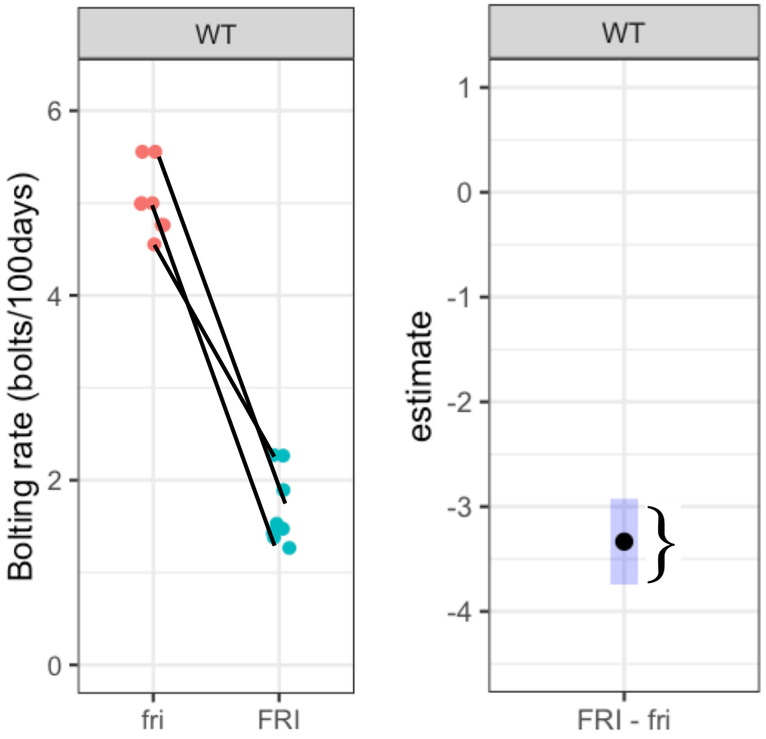
$$\text{Standard Error (SE)} = \sqrt{\frac{\text{Var(effect)} + \text{Var(measurement)}}{\text{\# replicates}}}$$

$$\text{Confidence Interval} = \text{Estimate} \pm SE * t_c$$

t_c = Critical value - dependent on Degrees of Freedom

Usually (# replicates - 1)

What determines the length of a Confidence Interval?



Variation in effect size

times we measure the effect

Measurement error per measurement

} Depends on **how** we use our data

Depends on **scope**

Option 1

Randomly pair FRI and fri plants, average the difference

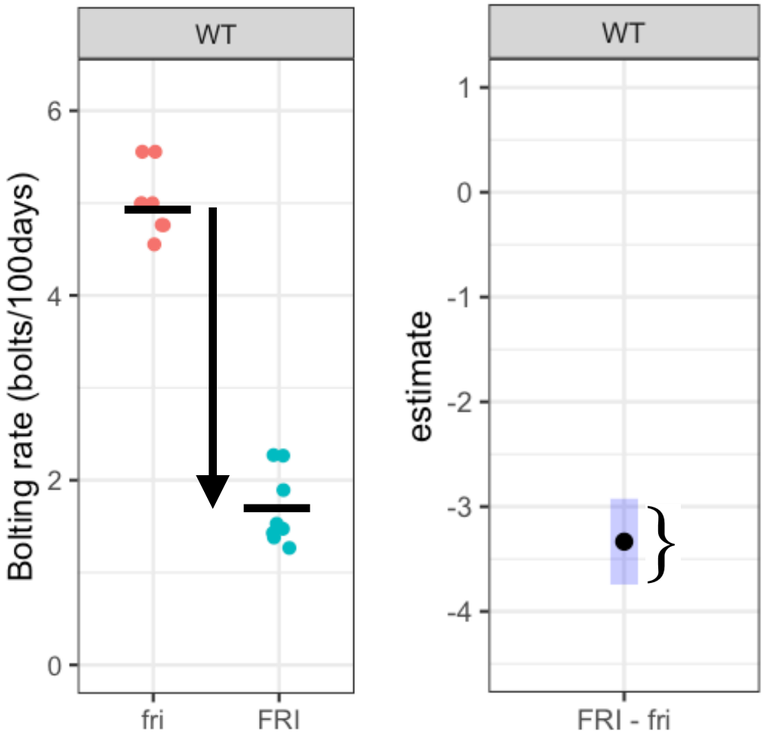
n(n-1)/2 pairs, but only (n-2) *independent* pairs

Option 2

Average FRI plants, and average fri plants, take the difference

only 1 measurement. But that measurement was more precise

What determines the length of a Confidence Interval?



Both give the **same estimate**

Different confidence intervals

Variation in effect size

times we measure the effect

Measurement error per measurement

} Depends on **how** we use our data

Depends on **scope**

Option 1

Randomly pair FRI and fri plants, average the difference

n(n-1)/2 pairs, but only (n-2) *independent* pairs

Option 2

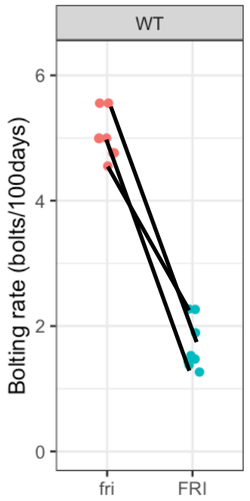
Average FRI plants, and average fri plants, take the difference

only 1 measurement. But that measurement was more precise

The difference depends on the **scope of inference**

Scope: How broad conclusions do we want to make?

n = 14



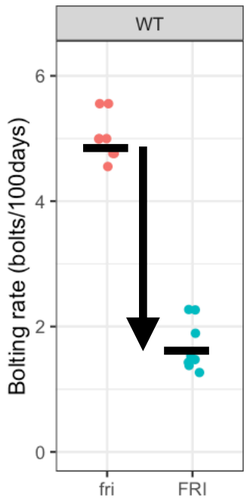
Option 1: Randomly pair FRI and fri plants, average the difference

scope: Average effect of FRI *in this condition in this experiment*

Randomly pairs are independent replicates within this experiment

narrow Confidence Intervals **Internal Validity**

n = 1



Option 2: Difference of averages

scope: Average effect of FRI *in conditions <u>similar to</u> this experiment*

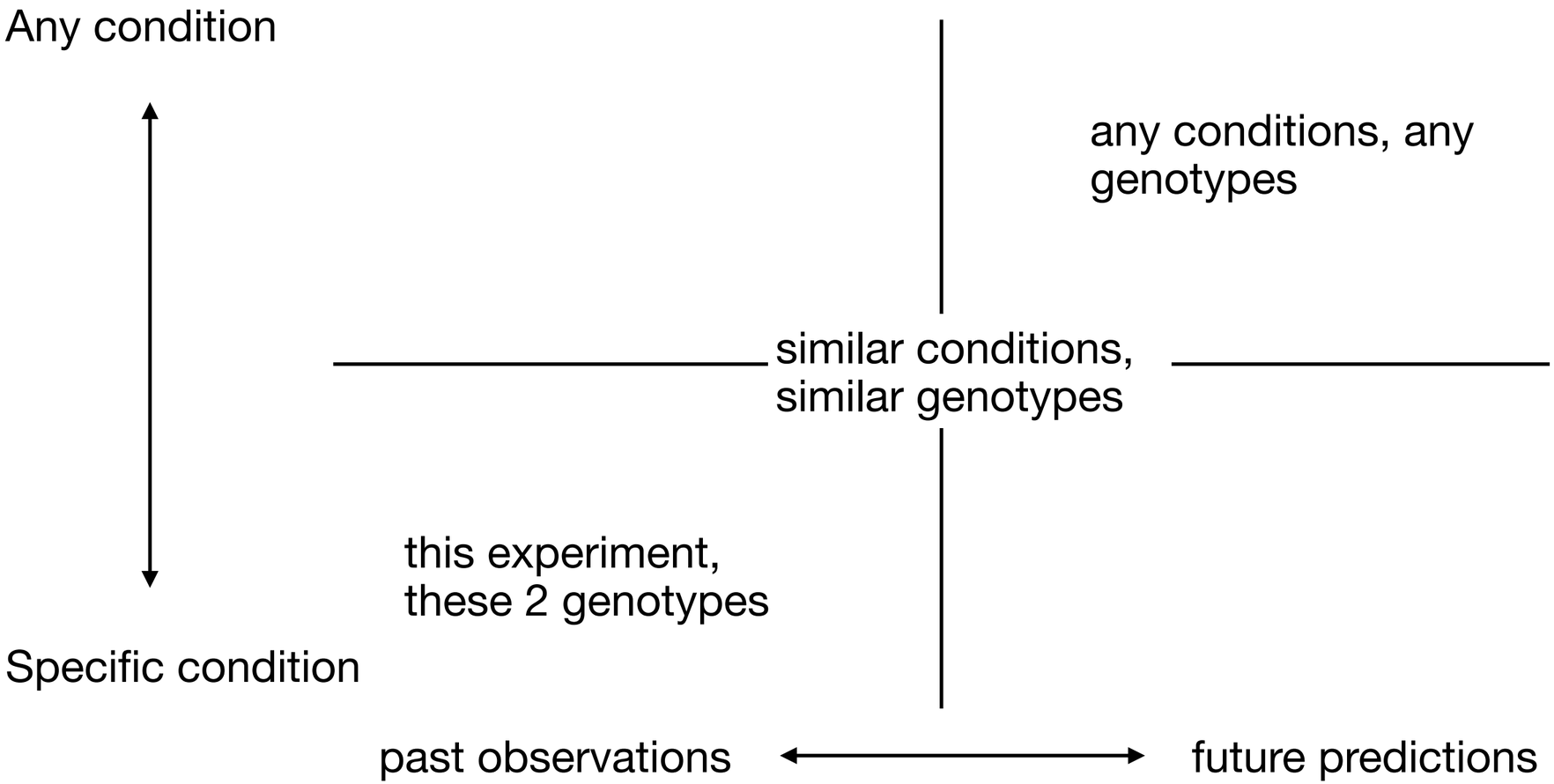
Randomly pairs are all still in <u>this particular condition</u>

We don't know how different they might be in a repeat of this experiment

wide Confidence Intervals **External Validity**

The difference depends on the **scope of inference**

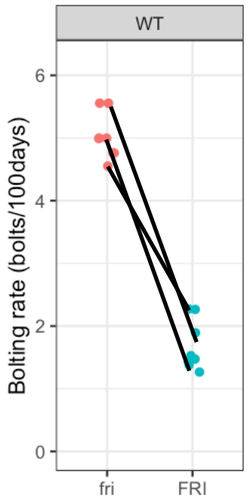
Scope: How broad conclusions do we want to make?



The difference depends on the **scope of inference**

Scope: How broad conclusions do we want to make?

n = 14



Option 1: Randomly pair FRI and fri plants, average the difference

scope: Average effect of FRI *in this condition in this experiment*

Randomly pairs are independent replicates within this experiment

narrow Confidence Intervals **Internal Validity**

Question: Is this really the effect of **FRI**?

Are the different FRI plants *independent?*

Scope: FRI gene **n=1**

Scope: *Col FRI* genotype **n=14**

What makes a replicate **independent**?

(# replicates) and (Degrees of Freedom) count **independent replicates**

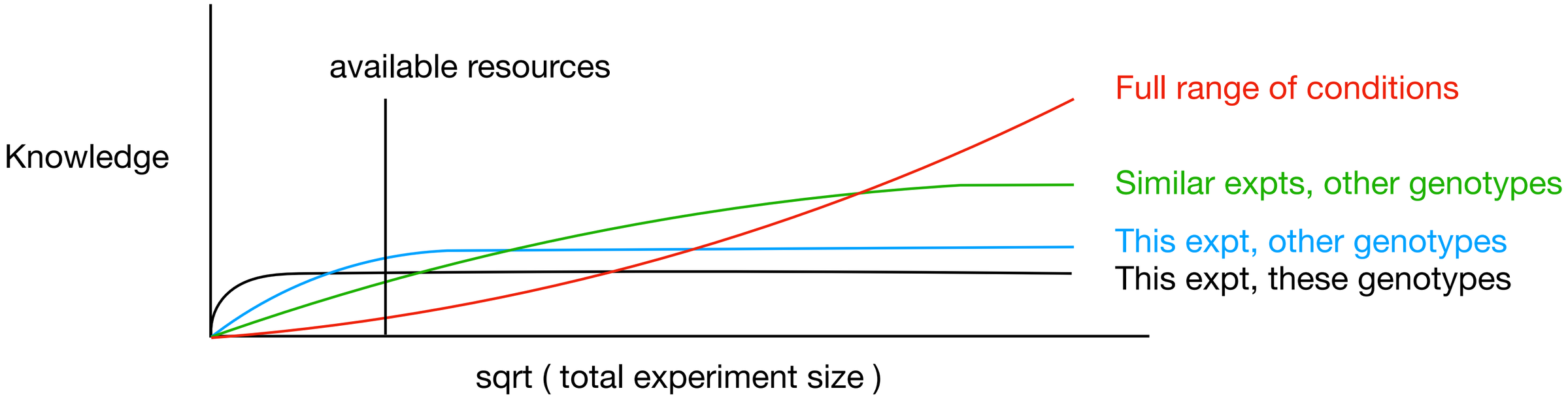
Essentially - each one is separately representative of the range of plausible individuals in the target population

Scope = this experiment, these 2 genotypes

Replicates compare Experimental Units

Scope = similar conditions, similar genotypes

Replicates are repeats of the experiment with newly constructed genotypes



Steps to analyze and experiment

1) Draw out the experimental layout

2) Create a **Design Table**

    Make decisions about scope and goals

3) Load data and check it against the Design Table

4) Create a **Model Statement**

5) Run analysis and make conclusions

Burghart *et al* 2016

Chamber 1

FRI fri

fri FRI

ConNV

Chamber 2

fri FRI

FRI fri

VarV

Chamber 3

FRI fri

fri FRI

ConV

Chamber 4

fri FRI

FRI fri

VarNV

Treatment	Block	EU
FRI	Chamber	Plant
Env	None	Chamber

Chamber 5

FRI fri

fri FRI

ConV

Chamber 6

fri FRI

FRI fri

ConNV

Chamber 7

FRI fri

fri FRI

VarNV

Chamber 8

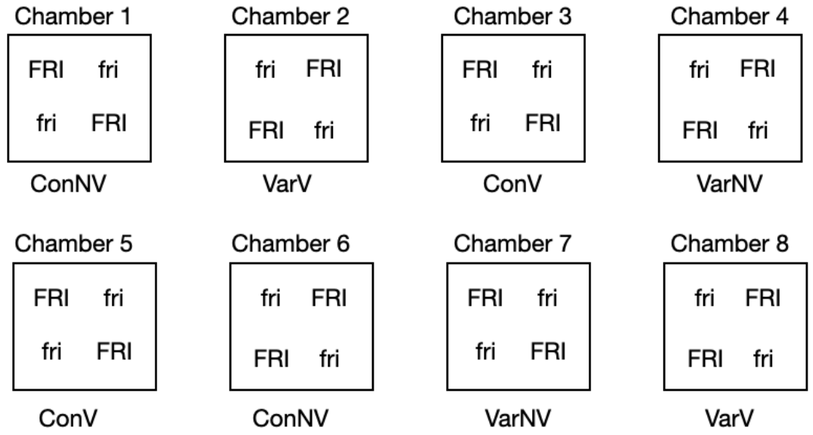
fri FRI

FRI fri

VarV

Design Table

Structure	Variable	# levels	Replicate	EU
Response	Days.to.Bolt	64		
focal treatment	FRI	2	Chamber	Plant
moderator treatment	Treatment.V	4	None	Chamber
combo treatment	FRI:Treatment.V	8	Chamber	Plant
Design	Chamber	8		
	Plant	64		
	FRI:Chamber	16		
	FRI:Treatment.V:Chamber	64		



Check data input in R

- Response is numeric
- Everything else is a factor
- Check # levels using str()

Model Statement

Structure	Variable	# levels	Replicate	EU
Response	Days.to.Bolt	64		
focal treatment	FRI	2	Chamber	Plant
moderator treatment	Treatment.V	4	None	Chamber
combo treatment	FRI:Treatment.V	8	Chamber	Plant
Design	Chamber	8		
	Plant	64		
	FRI:Chamber	16		
	FRI:Treatment.V:Chamber	32		

Response ~ Every other variable, separted by +

* except if # levels >= response

* except if two variables are **aliased**

use function is_aliased()

All EU declared as Random

(1|Plant) + (1|Chamber)

Block:Treatment declared Random for broader scope

Terms **nested** in Random terms are also Random

If any random terms, use *lmer()* instead of *lm()*

lmer(Days.to.Bolt ~ FRI + Treatment.V + FRI:Treatment.V +
(1|Chamber) + (1|FRI:Chamber) + (1|FRI:Treatment.V:Chamber))

Validity of conclusions

Are our estimates as good as they could be

Are we accuractly communicating the confidence we have in our conclusions?

Validity requires the correct pairing of Experimental Design, Analysis methods, and Conclusion statements

The same experiment can be valid or invalid depending on the analysis

The same analysis can be valid or invalid depending on the Conclusion statements

The same experiment can be validly analyzed in different ways depending on the scope

Internal Validity

Statements about the results of *this experiment*

Requires valid **Experimental Units**

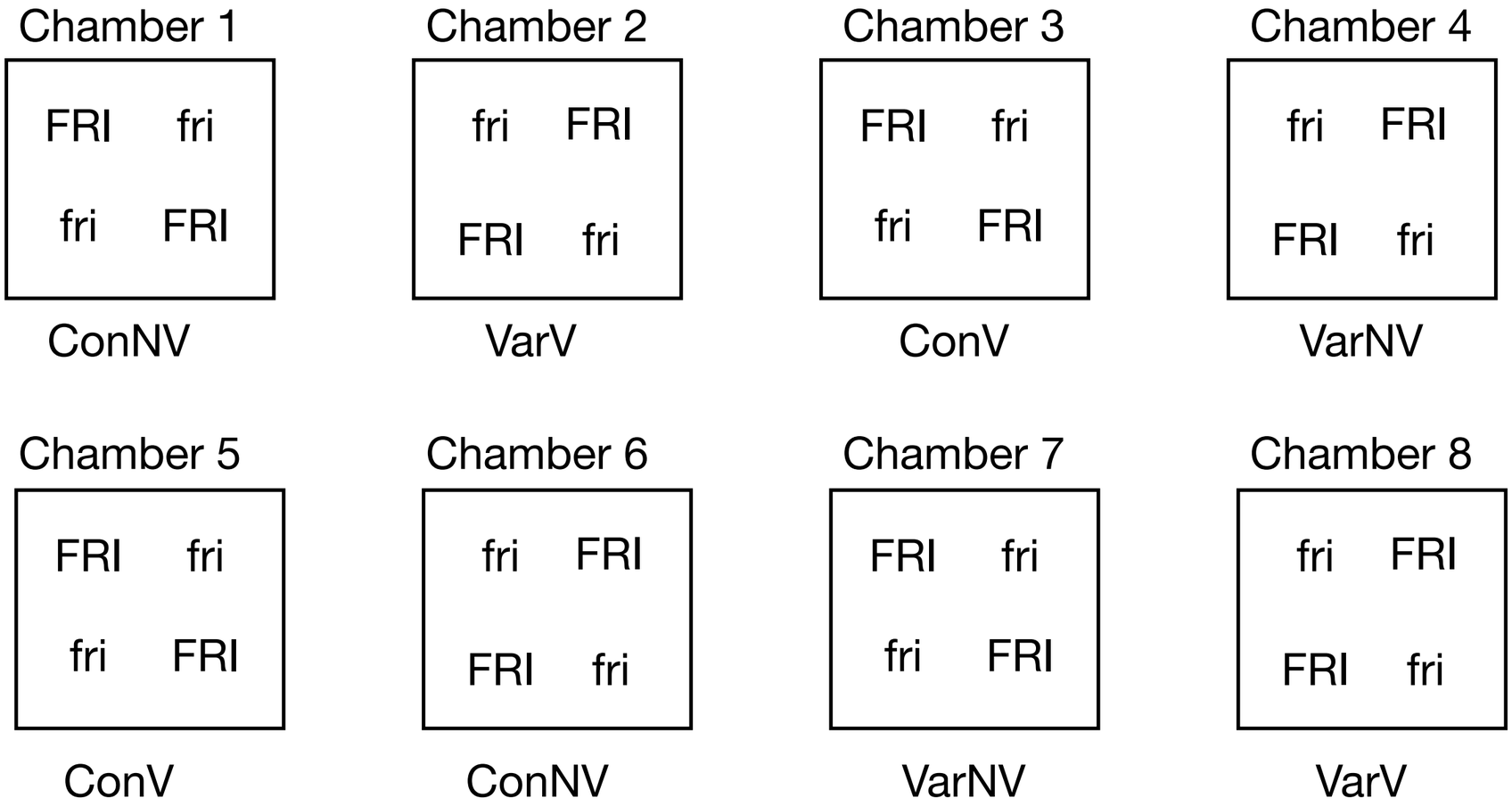
External Validity

Extrapolations to broader conditions

Requires valid **Experimental Units**

Requires valid **Replication**

Burghart *et al* 2016



What is the Experimental Unit for the **Environment** treatment? Chamber

What is the Experimental Unit for the **FRI** treatment? Plant

Analysis of experiments

Collecting data

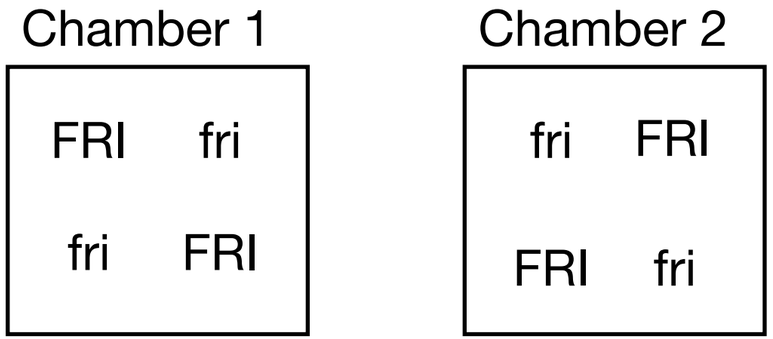
Loading data into R

Analyzing data

Reporting conclusions

Blocks

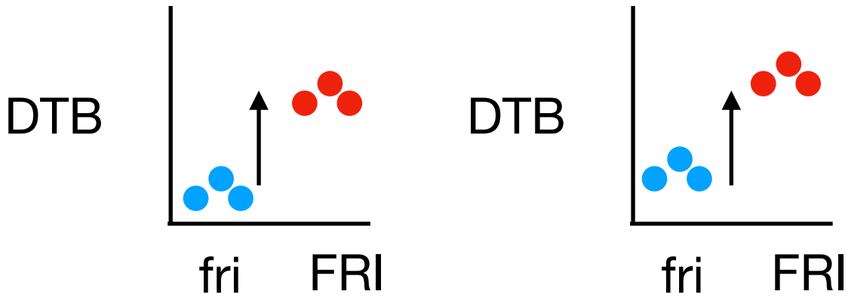
Block = mini experiment within a bigger experiment

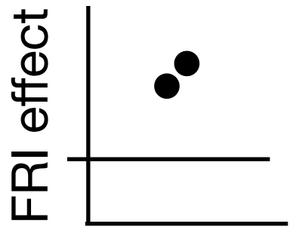


Each chamber has a **complete experiment**

1+ experimental units for 2+ treatment levels

We can measure the **treatment effect** within each chamber





Replicates of treatment **effects**

Necessary for **external validity**

Sometimes necessary / useful for interspersion (in

Chambers always differ *somewhat* from each other

FRI effects will be more similar within chambers than between chambers

Chambers always differ *somewhat* from each other

Blocks

Replicates of treatment **effects**

Necessary for **external validity**

Sometimes necessary / useful for interspersion (internal validity)

Block = mini experiment within a bigger experiment

Chamber 1

FRI	fri
fri	FRI

Chamber 2

fri	FRI
FRI	fri

Each chamber has a **complete experiment**

1+ experimental units for 2+ treatment levels

We can measure the **treatment effect** within each chamber

Chambers always differ *somewhat* from each other

FRI effects will be more similar within chambers than between chambers

Chambers always differ *somewhat* from each other

How do you replicate?

1. Repeat the whole experiment

   “Best” replication Necessary for treatments that cannot be interspersed

2. Form **Blocks** within your experiment

Chamber 1

FRI	fri
fri	FRI

Chamber 2

fri	FRI
FRI	fri

Chambers always differ *somewhat* from each other

If you have each genotype in each chamber, you can measure the FRI effect in each chamber

FRI effects **will differ** among chambers

If *FRI effects* **don’t change much**, you’ll have more confidence that it will replicate again the next time

If *FRI effects* **do change a lot**, you’ll know that the FRI effect is very sensitive

But you don’t know *why*

What is a Block?

Any grouping of Experimental Units of 2+ treatment levels

- Not all levels need to be in every block (but usually best if they are)

Best if the Experimental Units within the block are more similar than to other blocks

- This way you're exploring a greater range of conditions
- While comparisons within the block are still precise

A factor (e.g. a chamber) can be a block for one treatment, but an Experimental Unit for another!

Examples of common blocking factors

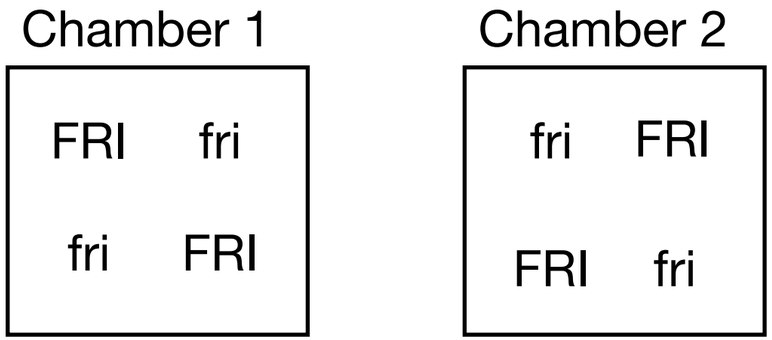
Chamber, petri dish	Plate
Field site	Undergrad technician
Year	Plant

How do you use blocks?

Experimental Design stage

Identify groups of Experimental Units that are similar

Randomize treatments within each block separately



How do you use blocks?

Analysis stage

Give each block a **unique name**

Declare the block variable in your model

Declare the block:treatment combination variable in your model too

Two choices **if you have 2+ experimental units / treatment level / block**:

1) Measure and report the treatment effects *separately in each block*

Small scope + block:treatment

2) Measure and report the *average treatment effect* (across blocks)

broad scope + (1|block:treatment)

Random Variable