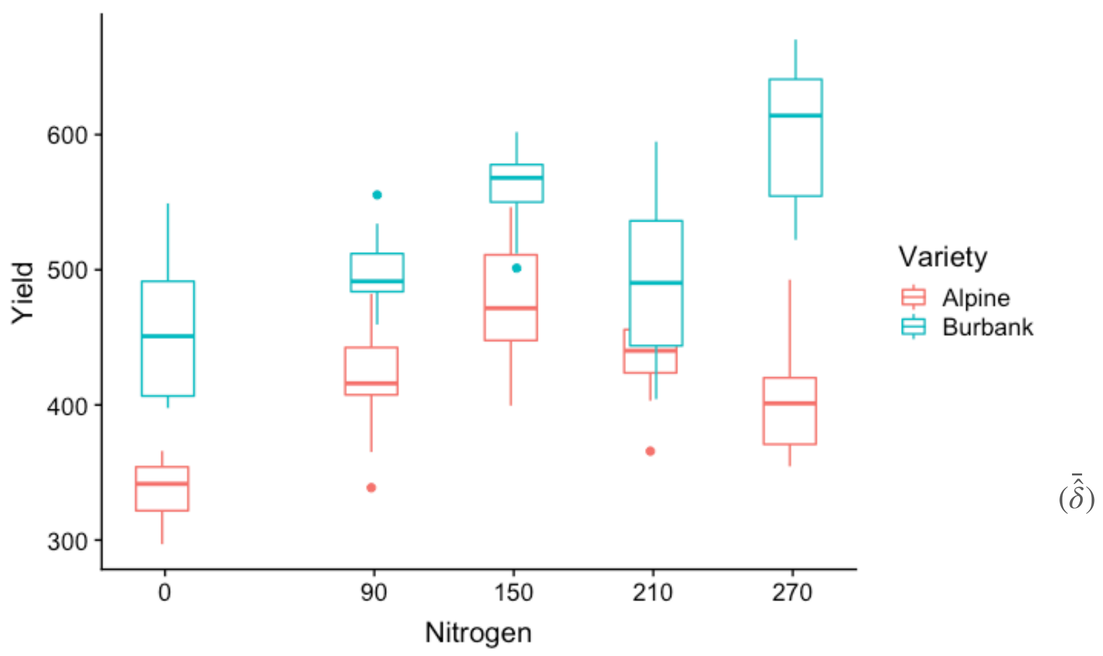
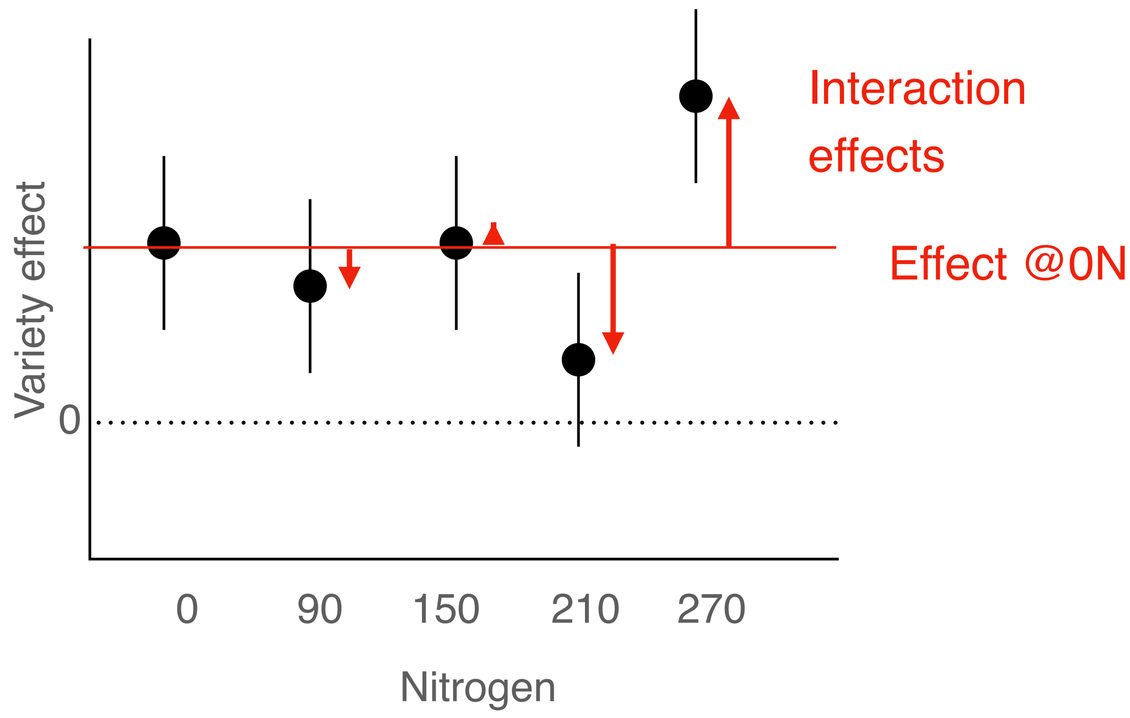
Does +Nitrogen **modify** the Variety effect?



$(\bar{\hat{\delta}})$



With 5 levels of Nitrogen, we can estimate the **Variety effect** (Burbank - Alpine) 5 times

Interaction effects How does the moderator **modify** the focal treatment's effect?

Changes in variety effects between levels of Nitrogen

- Dunnett: Do any Variety effects with +N differ from those with 0N?
- Tukey: Are any **pairwise contrasts** significant?
- ANOVA: Are all variety effects the same?

Does +Nitrogen **modify** the Variety effect?

Goal: Measure **Interaction Effects** (changes in Variety effects between levels of Nitrogen)

Treatment

Structure	Variable	Type	#levels	Replicate	EU
Focal	Variety	Cat	2	Nitrogen	Plot
Moderator	Nitrogen	Cat	5	None	Plot
Combos	Variety:Nitrogen	Cat	10	None	Plot
Design	Plot	Cat	100		
Response	Yield	Num	100		

Same table

Model

Drop all rows with #levels < # responses

Only EU need to be random. Treatment:Replicate **can** be random if not a treatment

For interaction effect ANOVA: **keep focal treatment variable!**

Doesn't matter for emmeans() analysis

lm(Yield ~ Variety + Nitrogen + Variety:Nitrogen)

Analysis

1) Fit model: lm() or lmer()

2) Model diagnostics: pls205_diagnostics(), specify EUs if they are a term in the model

3) (optional) ANOVA

```
Response: Yield
                 Df Sum Sq Mean Sq  F value    Pr(>F)
Nitrogen          4 167984   41996  21.0617 2.790e-12 ***
Variety           1 280604  280604 140.7279 < 2.2e-16 ***
Variety:Nitrogen  4  60001   15000   7.5229 2.831e-05 ***
Residuals        90 179456    1994
```

NumDF =
(# moderator levels - 1) *
(# Focal levels - 1)

Does +Nitrogen **modify** the Variety effect?

Goal: Measure **Interaction Effects** (changes in Variety effects between levels of Nitrogen)

Treatment

Structure	Variable	Type	#levels	Replicate	EU
Focal	Variety	Cat	2	Nitrogen	Plot
Moderator	Nitrogen	Cat	5	None	Plot
Combos	Variety:Nitrogen	Cat	10	None	Plot
Design	Plot	Cat	100		
Response	Yield	Num	100		

Same table

4) Estimate the Variety effects at each level of Nitrogen using emmeans() and contrast()

a) Calculate means for Variety at each level of Nitrogen

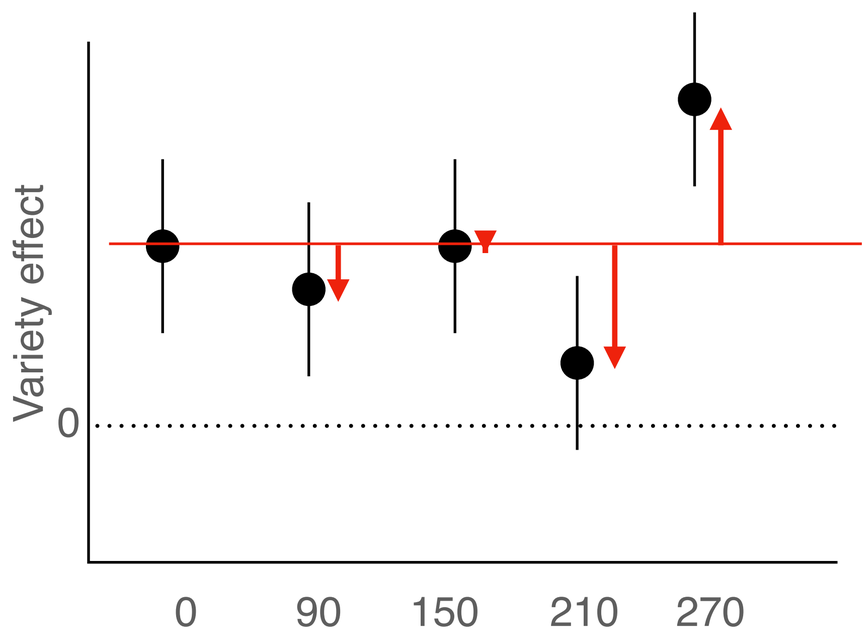
emmeans(model,specs = ‘Variety’, by = ‘Nitrogen’)

```
Nitrogen = 0:
 Variety emmean   SE df lower.CL upper.CL
 Alpine     345 14.1 90      316      373
 Burbank    455 14.1 90      427      483

Nitrogen = 90:
 Variety emmean   SE df lower.CL upper.CL
 Alpine     416 14.1 90      388      444
 Burbank    499 14.1 90      471      527
```

b) Contrast means within each Nitrogen level

effects = contrast(means,’pairwise’)

```
Nitrogen = 0:
 contrast          estimate SE df t.ratio p.value
 Burbank - Alpine     110.3 20 90   5.522  <.0001

Nitrogen = 90:
 contrast          estimate SE df t.ratio p.value
 Burbank - Alpine      82.3 20 90   4.120  0.0001
```



5) Contrast treatment effects (Burbank - Alpine) among levels of Nitrogen

regrouped_effects = update(effects,by= ‘contrast’)

contrast(regrouped_effects,’trt.vs.ctrl’)

```
contrast = Burbank - Alpine:
 contrast1                estimate   SE df t.ratio p.value
 Nitrogen90 - Nitrogen0      -28.0 28.2 90  -0.991  0.6906
 Nitrogen150 - Nitrogen0     -24.2 28.2 90  -0.856  0.7706
 Nitrogen210 - Nitrogen0     -56.3 28.2 90  -1.995  0.1565
 Nitrogen270 - Nitrogen0      86.9 28.2 90   3.077  0.0103

P value adjustment: dunnettx method for 4 tests
```

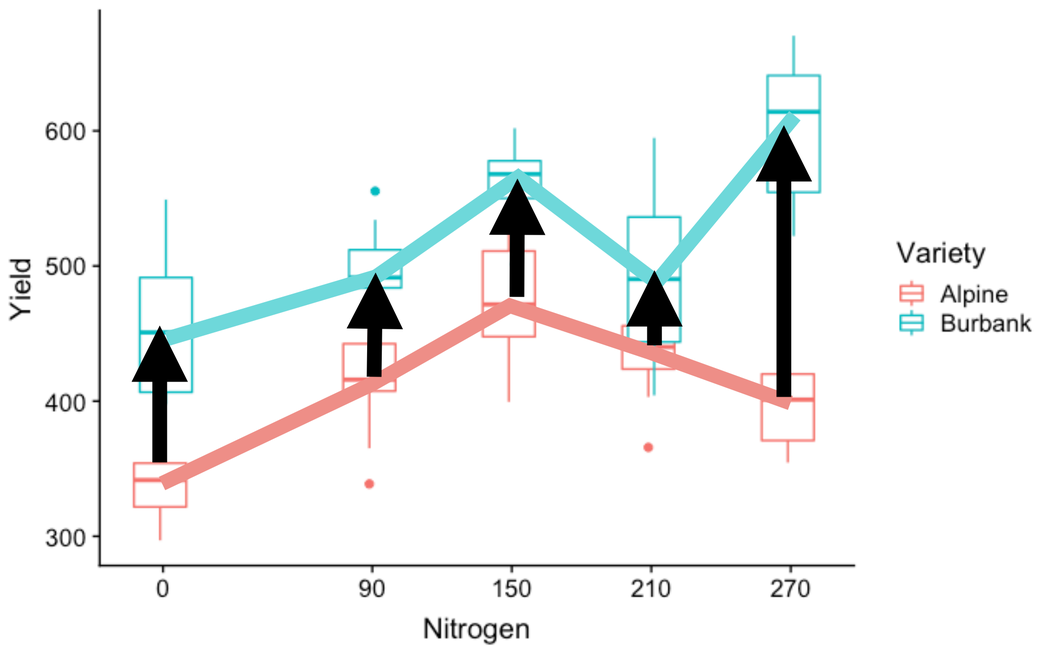
Visualizing Factorials

Treatment matrix:

Variety	Nitrogen 0	90	150	210	270
Alpine	10	10	10	10	10
Burbank	10	10	10	10	10

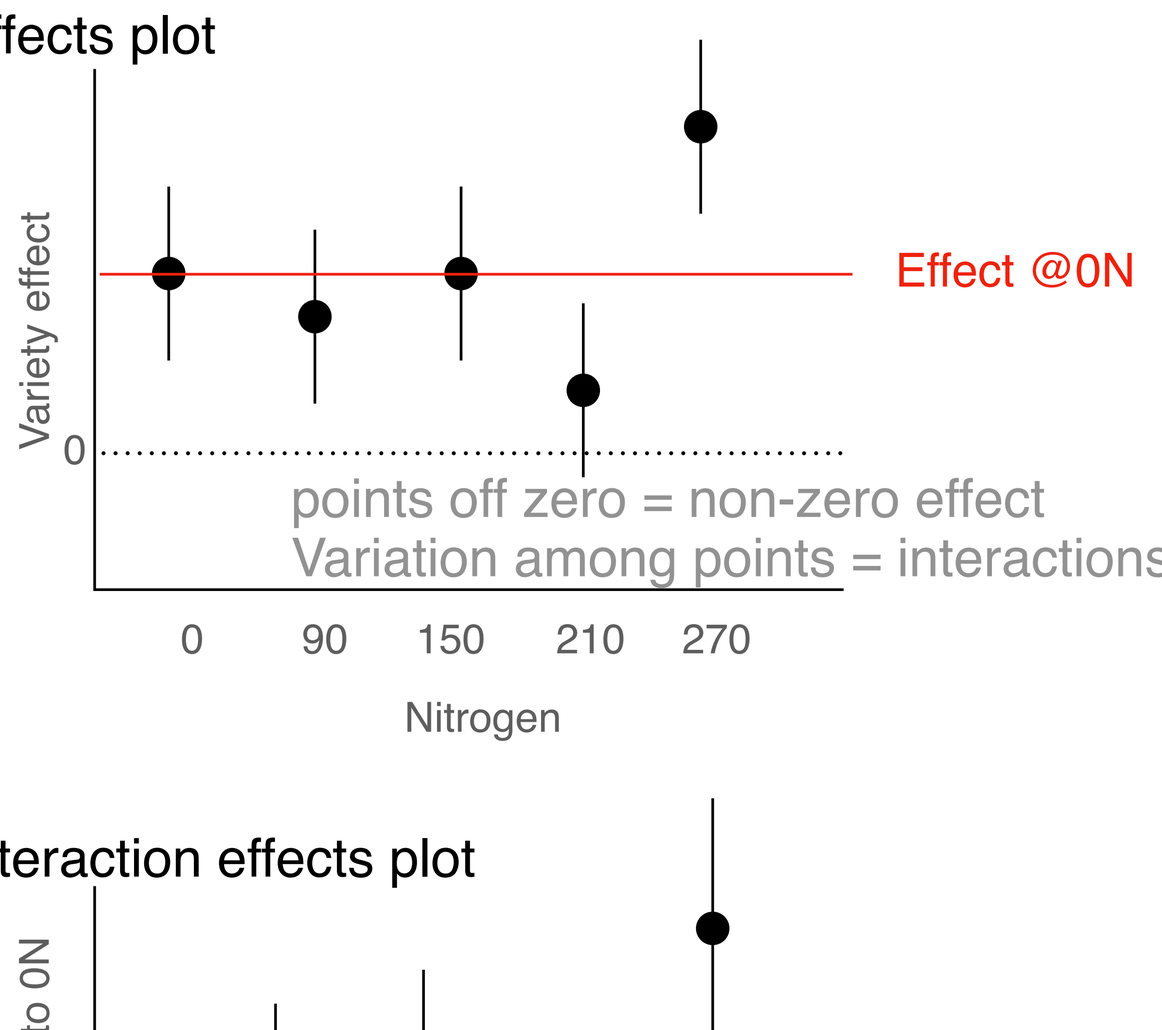
plots / treatment combo

Interaction plot

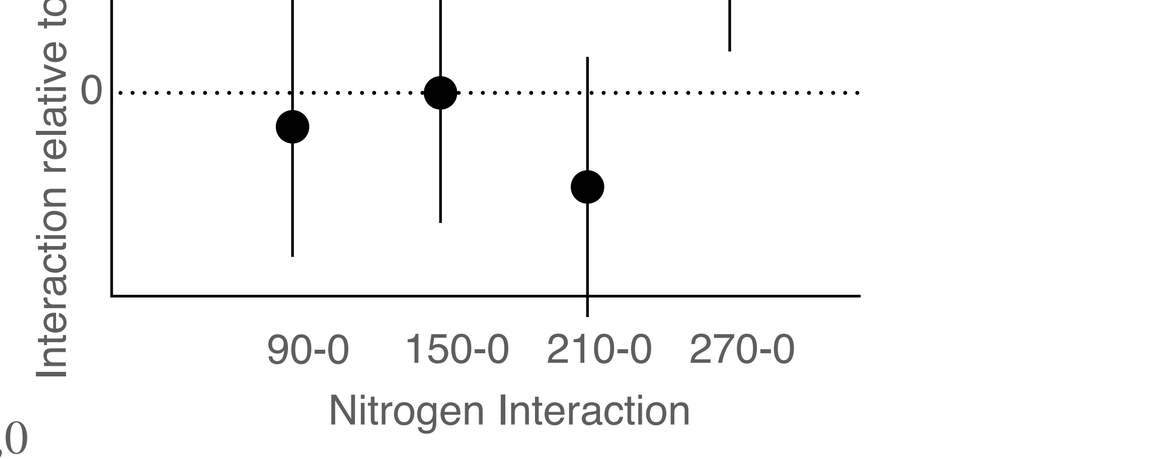


parallel lines = no interaction

Effects plot



Interaction effects plot



$\mu_{B,0} - \mu_{A,0}$

Specific effects **vs** Interaction effects

Variety effect @ 0N?

Average effect of changing from Alpine to Burbank in plots with 0N

We estimate average **yields** of Alpine @0N and Burbank @0N

Indirect experiment: $\hat{\mu}_{B,0} - \hat{\mu}_{A,0} = \hat{\delta}_0$

Strategy: emmeans() to calculate $\hat{\mu}_i$, contrasts() to calculate $\hat{\delta}$

Effect of changing from 0N to 90N on difference between varieties?

We estimate average **Variety effects** @0N and @90N

Indirect experiment: $\hat{\delta}_{90} - \hat{\delta}_0 = \hat{I}_{90-0}$

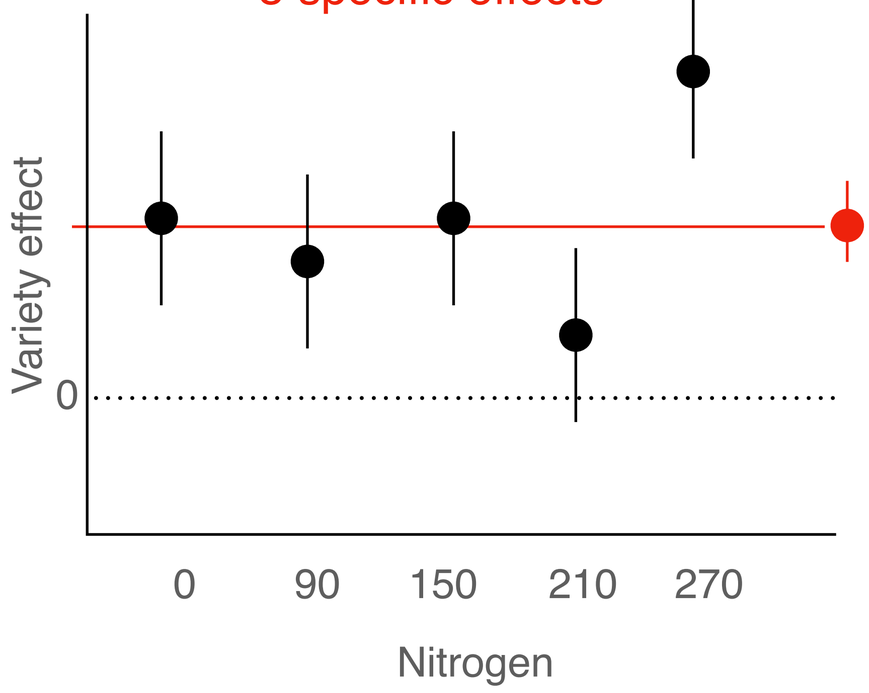
Strategy: contrast() to calculate $\hat{\delta}_i$, contrasts() to calculate $\hat{I}$

What about M**ain Effects**?

Most textbooks spend a lot of time on main effects vs interactions for factorials

```
Response: Yield
                 Df Sum Sq Mean Sq  F value    Pr(>F)
Variety           1 280604  280604 140.7279 < 2.2e-16 ***
Nitrogen          4 167984   41996  21.0617 2.790e-12 ***
Variety:Nitrogen  4  60001   15000   7.5229 2.831e-05 ***
Residuals        90 179456    1994
```

Main Effects are the average of the specific effects



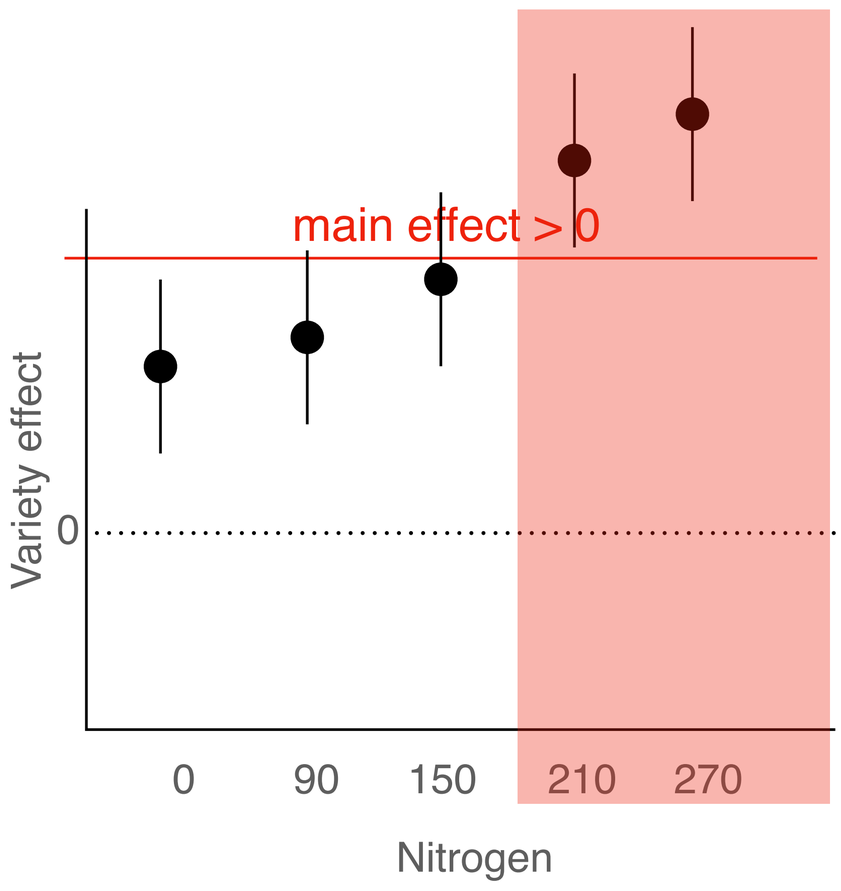
What is the SE of the Main Effect?

$$\sqrt{\frac{\sigma_r^2(\hat{\mu}_{ij})}{50} + \frac{\sigma_r^2(\hat{\mu}_{ij})}{50}}$$

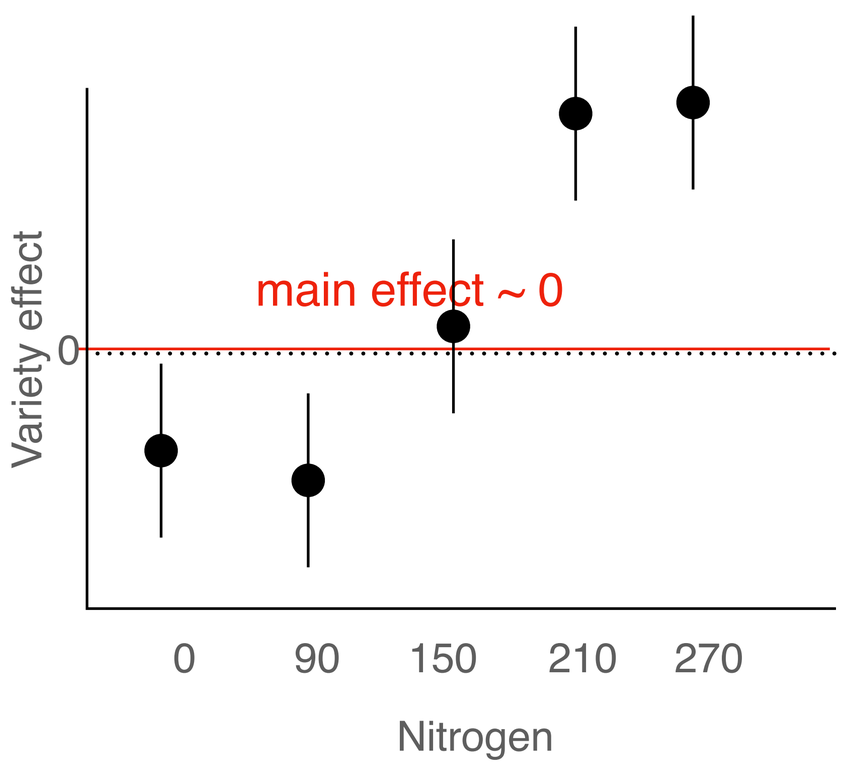
SE for the average of estimates of **THESE 5 levels**

$$\sqrt{\frac{\sigma_r^2(\hat{\delta}_i)}{5}}$$

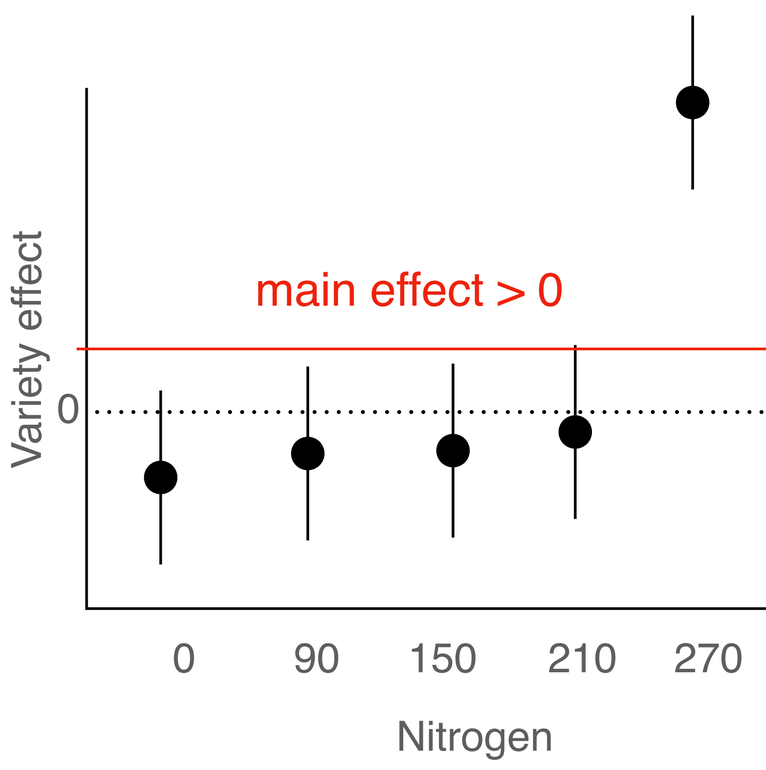
SE for the average of 5 estimates



What if no farmer would use > 150?



most specific effects are large



main effect is highly misleading

Main effect ~0 doesn't mean Variety doesn't have an effect

Main effect > 0 doesn't mean that Variety usually has a positive effect

To do the correct analysis of main effects, you must declare (1|Variety:Nitrogen) in your model

Three-way factorials!

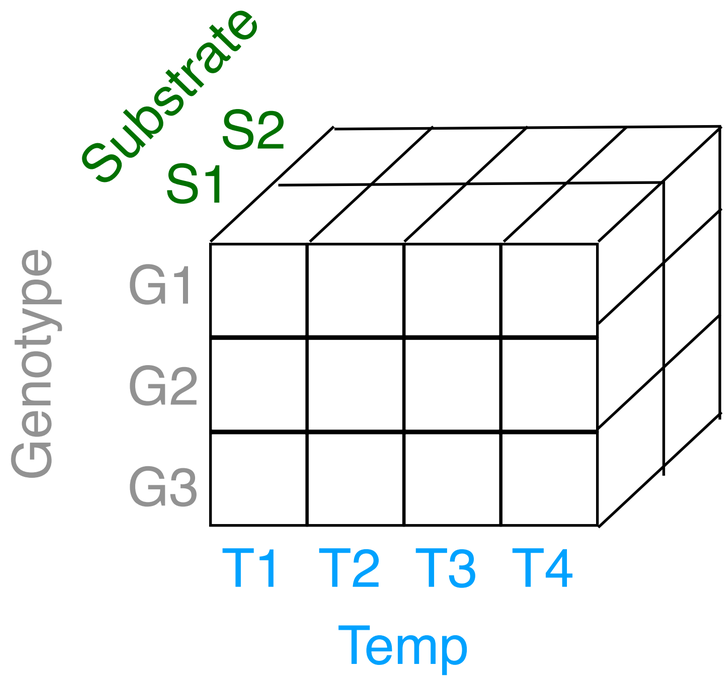
An experiment is interested in the different activity levels of three strains (Genotype) of beetle

- Activity is strongly affected by temperature, so she chooses 4 temperatures

- Since the mutation affects coloration, the substrate (grass or dirt) may be important

She takes 24 beetles of each genotype, and randomly allocates each to one of the combinations of Temp or Substrate

What is the EU for this experiment?



3x4x2 Factorial

24 treatment combinations: G1:T1:S1, G1:T2:S3,…

Focal treatment(s)?

Moderator treatment(s)?

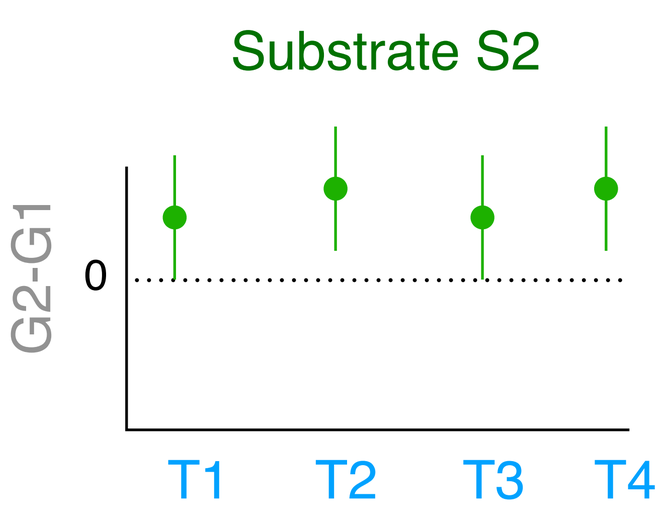
Specific effects

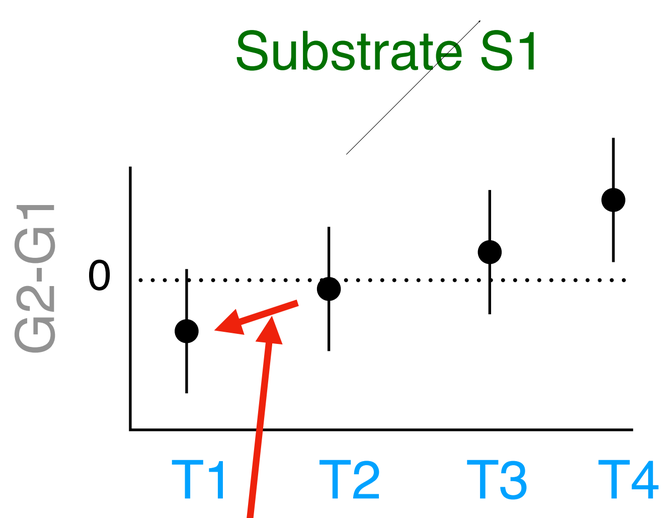
Interaction effects

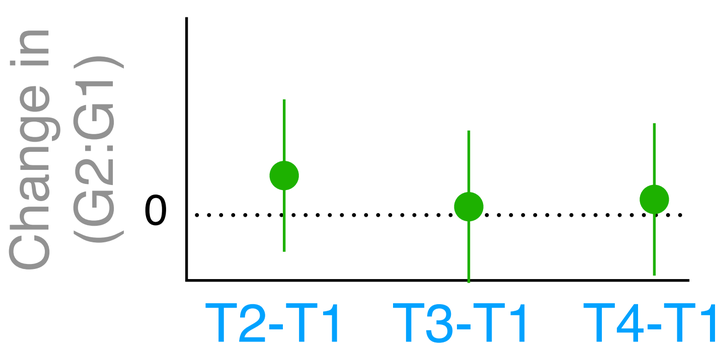
Focal: Substrate

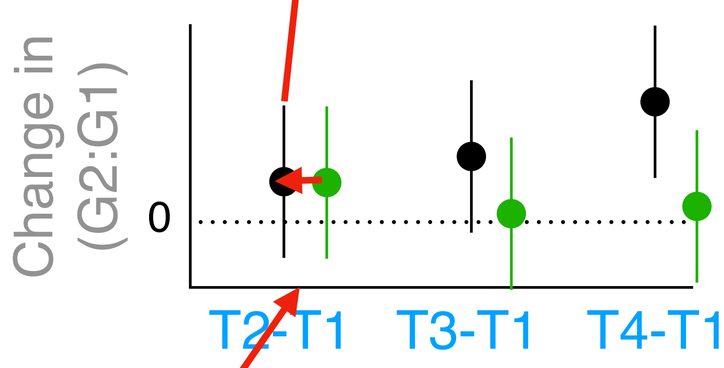
Moderator1: Genotype

Moderator2: Temp









Specific effects:

Differences among genotypes at each Temp

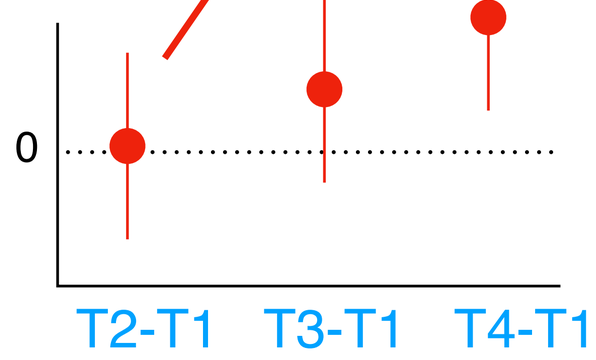
…for each substrate

2-way Interaction effects:

Change in genotype effects **among different Temps**

…for each substrate

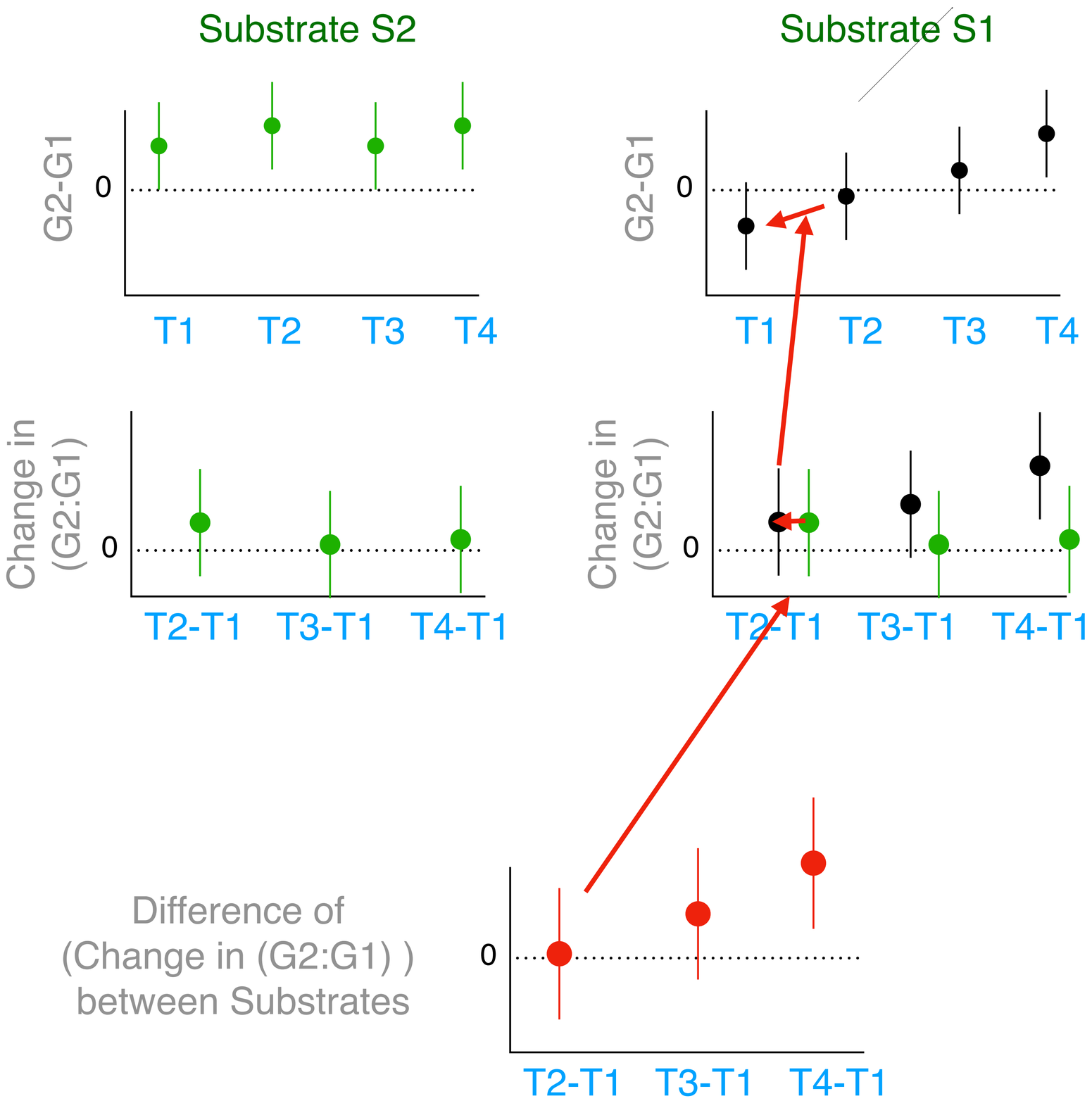
Difference of (Change in (G2:G1)) between Substrates



3-way Interaction effects:

How the “change in genotype effects among different Temps” **differs depending on Substrate**

Three-way interactions are hard to interpret



Specific effects:

Differences among genotypes at each Temp

…for each substrate

2-way Interaction effects:

Change in genotype effects **among different Temps**

…for each substrate

3-way Interaction effects:

How the “change in genotype effects among different Temps” **differs depending on Substrate**

Geno:Temp:Substrate

Not: Does Genotype interact with Temp **or** Substrate

Not: Do specific combinations of Temp and Substrate alter Genotype effects

Not: Does Genotype matter in any Substrate and/or Temperature

Yes: How different are the temperature effects on genotype differences between substrates?

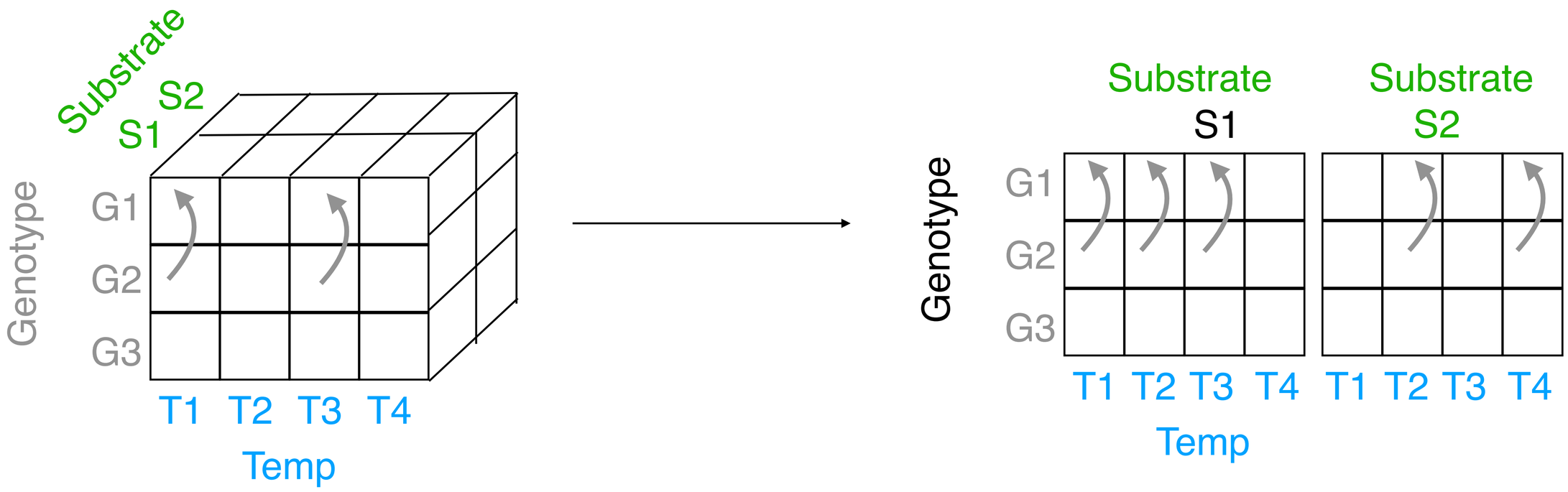
3-way (or more) factorials are very common. WHY?

Solution: Think of them as 2-way factorials

1) Think of them as a big 2-way factorial by combining moderators into 1 treatment

2) Think of the **focal effect** as the response, and **moderator 1** as the focal

Three-way factorial as a big 2-way factorial



1) Does Genotype have an effect in **any combination of Temp and Substrate?**

focal: Genotype moderator: Temp:Substrate

strategy1: create new variable Temp_Substrate

strategy2: use by = c('Temp','Substrate') in emmeans()

correct for number of levels of Temp:Substrate

2) Does any combination environment (combination of Temp and Substrate) modify Genotype effects?

focal: Genotype moderator: Temp_Substrate

Treatment

Structure	Variable	Type	#levels	Replicate	EU
Focal	Genotype	Cat	3	Temp:Substrate	Beetle
Moderator	Temp:Substrate	Cat	8	None	Beetle
Combos	Geno:Temp:Subst	Cat	24	None	Beetle
Design	Beetle	Cat	72		
Response	Activity	Num	72		

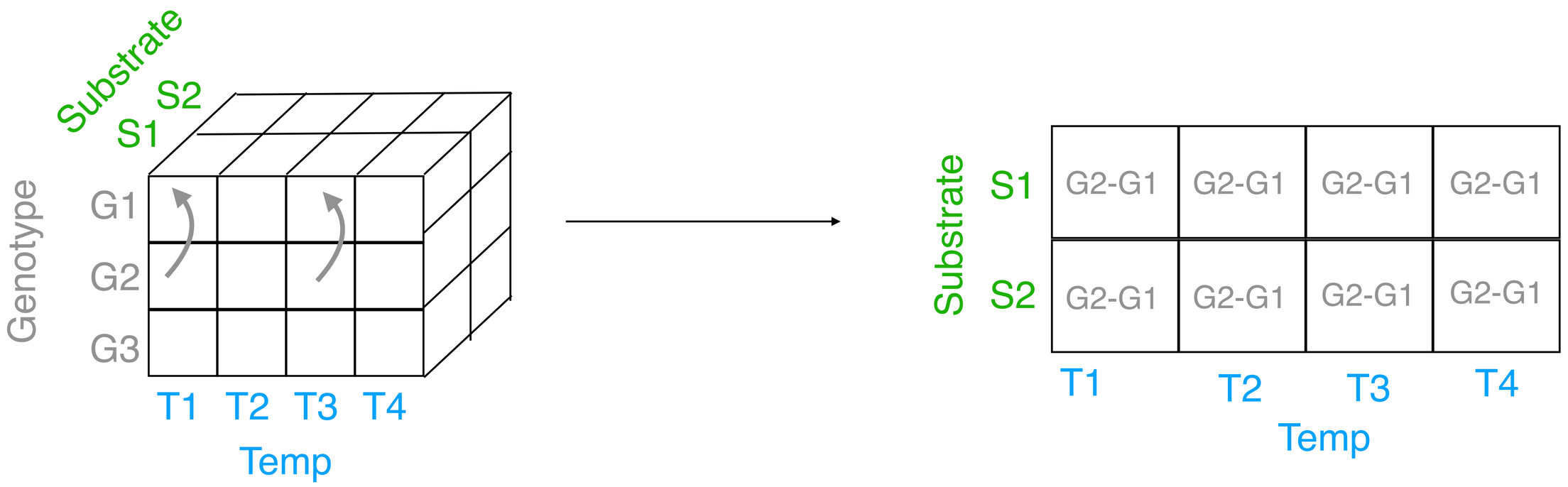
* This works for emmeans(), for ANOVA you need to create "Temp_Substrate"

Write the model for 1 (Specific Effects) and 2 (Iteractions)

1) lm(Activity ~ Temp_Substrate + Geno:Temp_Substrate)

2) lm(Activity ~ Geno + Temp_Substrate + Geno:Temp_Substrate)

Three-way factorial as 2-way with Geno_effect as the response



Think of the “Genotype effect” as a property of a beetle.

“What happens to it when you mutate a specific gene?”

Like: “What does your pulse do when you stand up”?

We could imagine measuring this on each beetle

Think of this as the response

Substrate effect (new focal treatment)

Do we see the effect of mutations in some substrates but not others?

Specific effects: Is there a substrate effect on the mutation at any temperature?

Interaction effect: Do we see substrate effects in some temperatures more than others?

Strategy

1) Estimate Genotype effects (G2-G1) in each combo of Temp and Substrate

emmeans() -> contrast()

2) Treat these effects as you would focal treatment means

1) Calculate Specific Effects of Substrate (focal) on these estimates (with by = ‘Temp’)

2) Calculate Interaction Effects of Temp:Substrate by regrouping and contrasting the specific effects

3) Report Specific effects and/or Interactions on this new trait: “Geno_effect”

ANOVA

Identify the analysis represented by each ANOVA table:

Write out a statement in words **without using the word "Interaction"**

We only look at the last row!

```
Response: Activity
                    Df Sum Sq Mean Sq F value    Pr(>F)
Temp                 3  27270  9090.1 11.4686 8.671e-06 ***
Geno                 2   2365  1182.6  1.4920 0.2351727
Substrate            1  11167 11167.3 14.0892 0.0004706 ***
Temp:Geno            6  24834  4138.9  5.2219 0.0003319 ***
Temp:Substrate       3  18170  6056.6  7.6413 0.0002831 ***
Geno:Substrate       2  10025  5012.4  6.3239 0.0036494 **
Temp:Geno:Substrate  6    157    26.2  0.0330 0.9998321
Residuals           48  38045   792.6
```

3-way interaction

How temperature modifies the effect of substrate on how much Genotype matters for a beetle

```
Response: Activity
                    Df Sum Sq Mean Sq F value    Pr(>F)
Geno                 2   2365  1182.6  1.4920  0.235173
Temp_Substrate       7  56607  8086.8 10.2027 9.467e-08 ***
Geno:Temp_Substrate 14  35015  2501.1  3.1555  0.001507 **
Residuals           48  38045   792.6
```

Big 2-way interactions

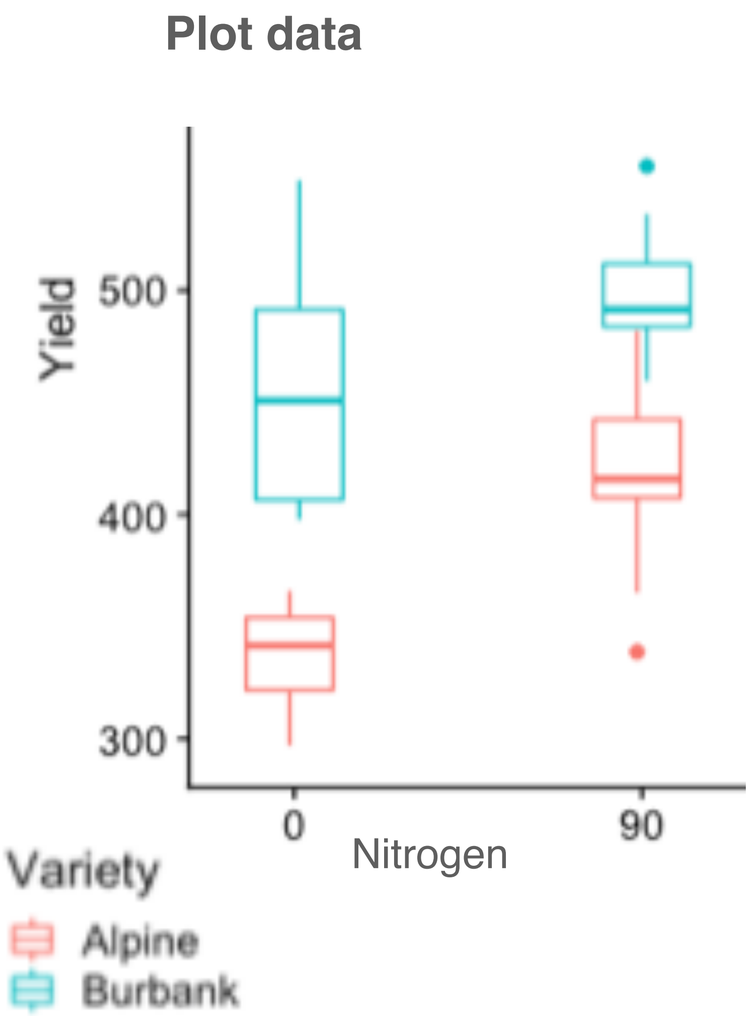
Do any combinations of Temp and Substrate alter the Genotype effects?

```
Response: Activity
                    Df Sum Sq Mean Sq F value    Pr(>F)
Temp_Substrate       7  56607  8086.8 10.2027 9.467e-08 ***
Temp_Substrate:Geno 16  37380  2336.3  2.9476  0.001942 **
Residuals           48  38045   792.6
```

Big 2-way specific effects

Does Genotype matter in any combination of Temp or Substrate?

Interaction effects in a factorial



Define treatments of interest

focal = Variety, moderator = Nitrogen

Calculate effects of focal **at each level** of moderator

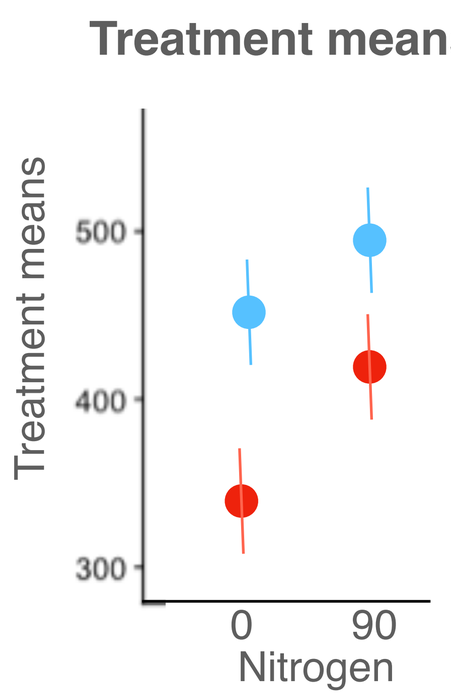
Calculate moderator effect **on** focal effects

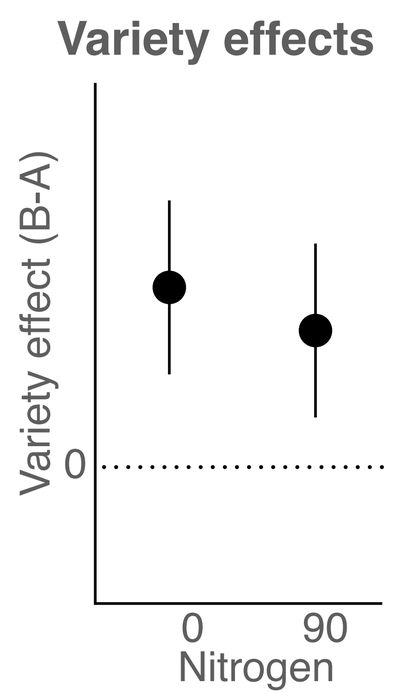
This is the Interaction

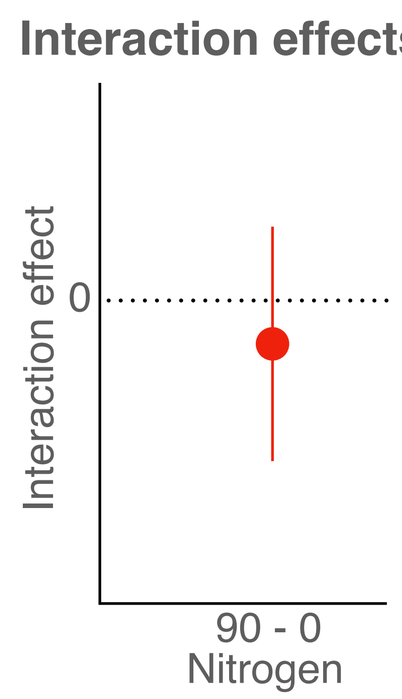
Notice what happens to the SE calculations!

points = EU estimates

$$\sigma^2_{\hat{\mu}_i} = \sigma^2_{\mu_{ij}} + \sigma^2_m$$







	Ave value of treatment combination	**Effect** of focal trt at each level of mediator	**Effect** of mediator on focal effect
estimate	$(\hat{\mu}_i)$ mean of plots	$(\hat{\delta}_j)$ difference between trt means	$(\hat{I}_k)$ difference between variety effects
SE	$\sqrt{\sigma^2_{\hat{\mu}_i}/n_i} = \sqrt{\sigma^2_r(\hat{\mu}_i)}$	$\sqrt{\sigma^2_r(\hat{\mu}_1) + \sigma^2_r(\hat{\mu}_2)} = \sqrt{\sigma^2_r(\hat{\delta}_j)}$	$\sqrt{\sigma^2_r(\hat{\delta}_1) + \sigma^2_r(\hat{\delta}_2)}$
	averaging $n_i = 10$ plots	averaging $n_j = 1$ treatment means	