### Incomplete block designs

Goal: smaller blocks can be more homogeneous

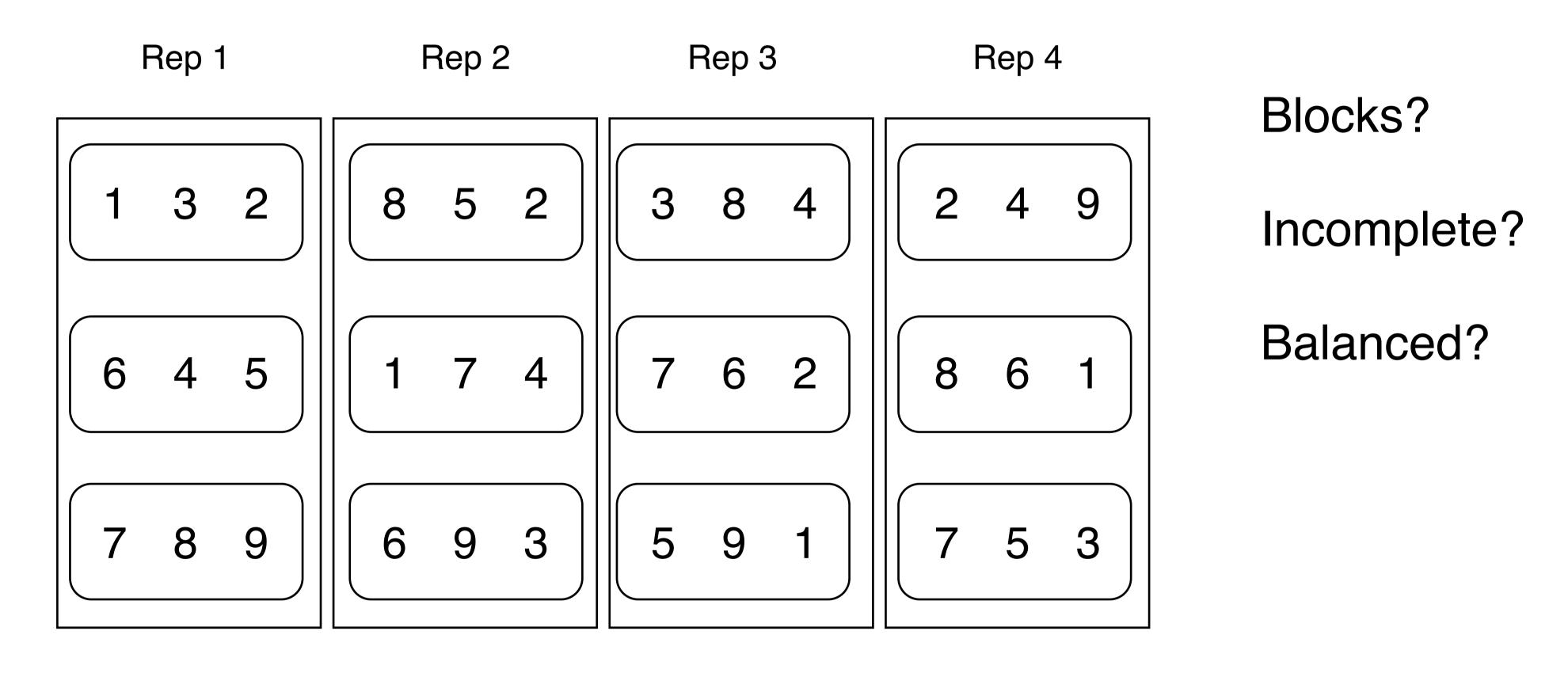
Strategy: Think about balance and connectedness

Analysis:

Put Blocks first in formula. Consider declaring Block to be random

No change in emmeans

Use emmeans before experiment to check connectedness



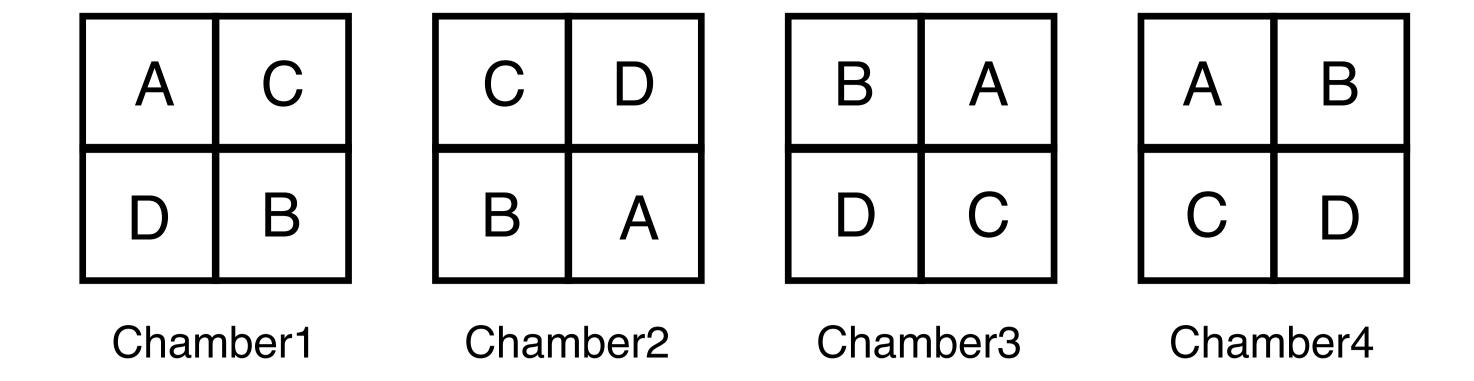
Resolvable Incomplete Block Design

Resolvable: each Rep is a complete block

Can analyze each Rep separately, combine. Or drop an entire rep.

An experiment was run to compare 4 tomato genotypes for their flowering times

The experiment was done in growth chambers with 1 plant of each in each chamber



What type of experiment is this?

What is the EU?

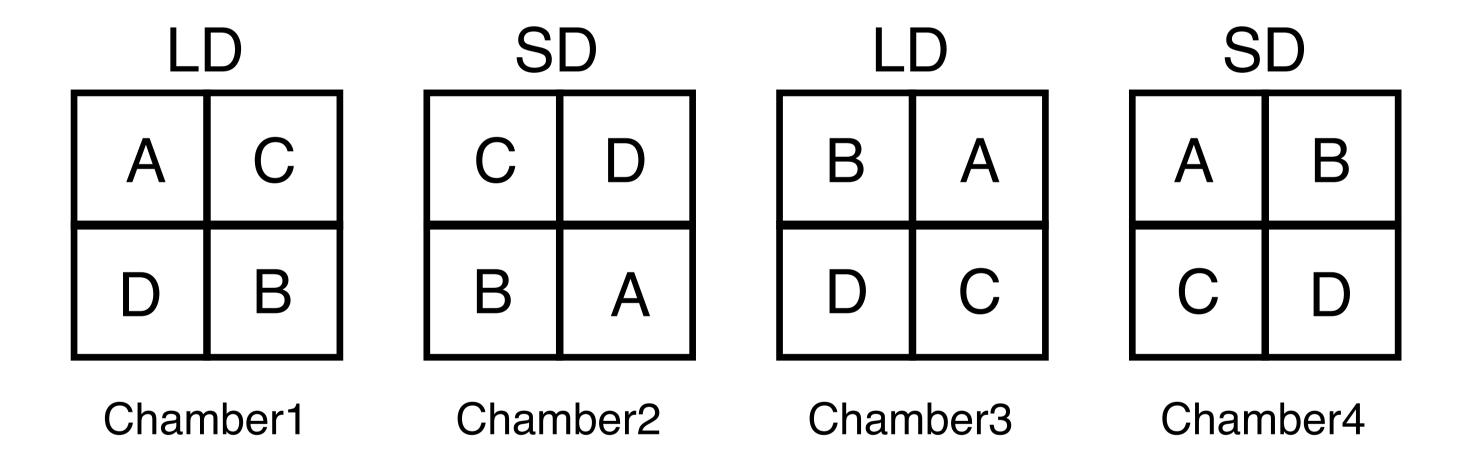
What estimates does the researcher want to report?

An experiment was run to compare 4 tomato genotypes for their flowering times

The experiment was done in growth chambers with 1 plant of each in each chamber

The experimenter was interested if daylength altered the flowering differences among genotypes

Two chambers were set to 16h days (Long days = LD) and two to 8h (Short days = SD)



What type of experiment is this?

Factorial? RCBD?

Split-plot factorial

What is the EU?

Depends on the treatment

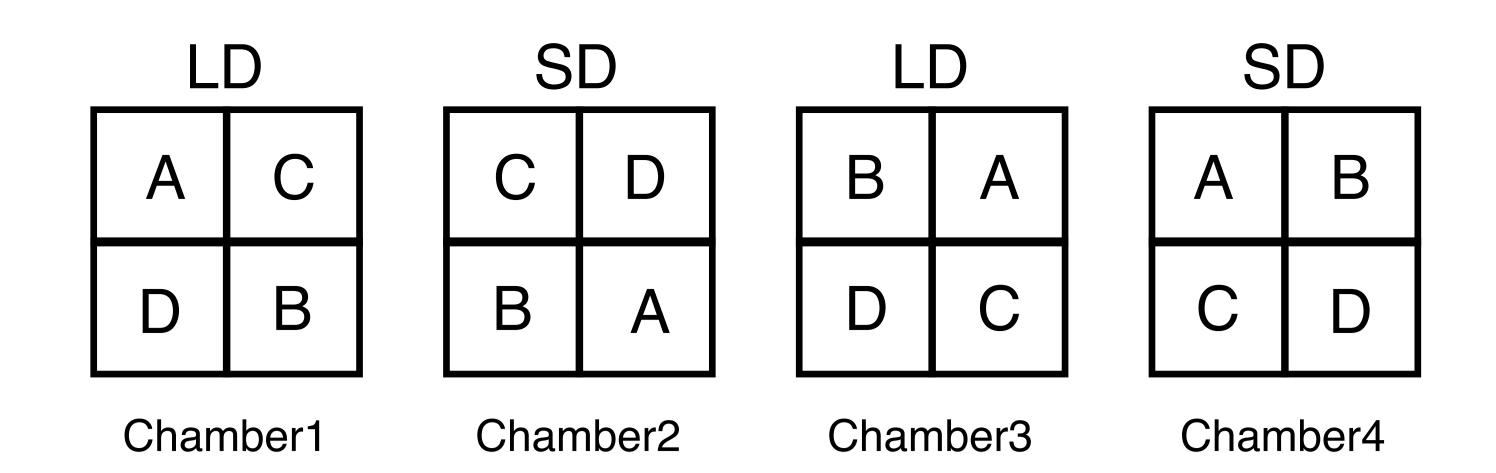
What estimates does the researcher want to report? focal = Genotype moderator = Daylength

Specific effects (Genotype effects at each Daylength)

Interaction effects (Daylength effects on Genotype differences)

What are the consequences of the Split-plot design for the analysis?

Multiple EU, different SEs for different types of effects



Split-plot design

Idea

Design used for factorial experiments

Two-stage randomization

We can use chambers as blocks for Geno but not for Daylength

1) Daylength treatments randomized to chambers

We get some benefit from blocking one treatment vs neither

- 2) Genotypes randomized to pots within each chamber

Naming:

Chambers = Main Plots

Plant = Sub Plots / Split-plots

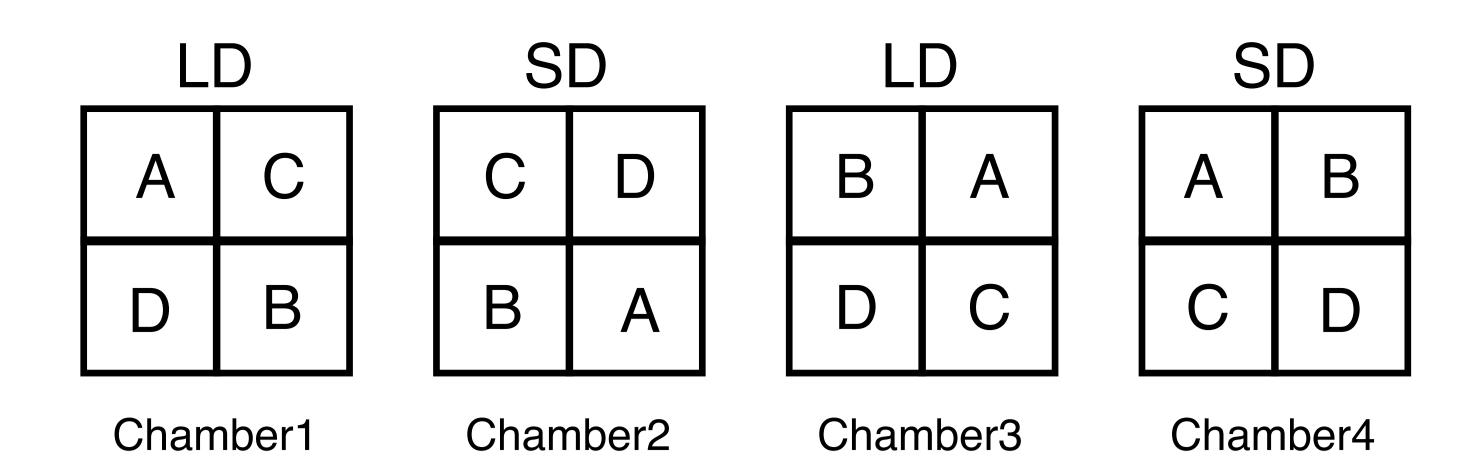
EUs:

Different for each treatment variable Be sure to declare them all!

Blocks:

Different for each treatment variable Be sure to declare them all!

Three experiments										EU	J?	Block?	
1) Genotype	A C		С	D	В	Α	Α	В					
	D	В		В	Α	D	С	С	D	Pla	nt	Chamber	
	Chaml	ber1		Chamb	er2	Chamb	per3	Chamb	er4				
2) Daylength	Cham	D —		—S Chaml		L	D ——ber3	—S Chamb		Cha	amber	None	
3) Genotype:Daylength		LDC		SDB Chamb	SD A	LDB	<sub>LD</sub> C	SDA SDC	<sub>SD</sub> D	Plan	nt	Chamber Incomplete Blo	ock



Focal: Geno Moderator: Daylength

Structure	Variable	#levels	Block	Experimental Unit
Focal	Geno	4	Dayl, Chamber	Plant
Moderator	Dayl	2	None	Chamber
Combo	Geno:Dayl	8	Chamber	Plant
Design	Chamber	4		
	Plant	16		
	Geno:Chamber	16		
	Geno:Dayl:Chamber	16		
Response	Days	16		

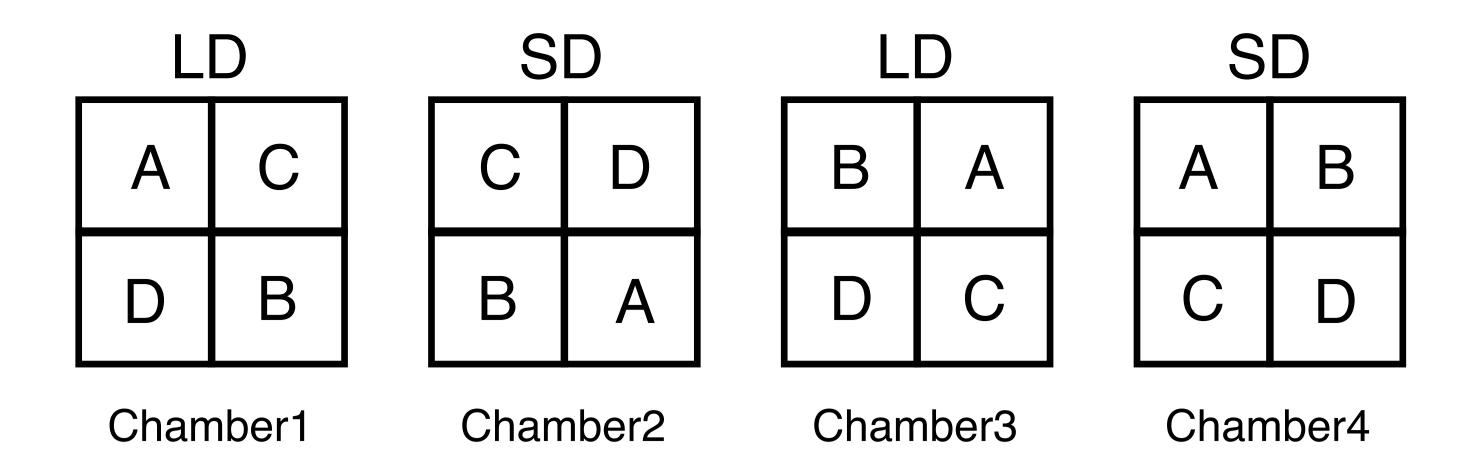
Imer(Days~Geno+DayI+Geno:DayI+(1|Chamber))

	level relationships	Keep term?	Make combinations?
aliased	levels are 1:1	only one of set	No
nested	1-many	all	No
crossed	many-many	all	Yes

Repeat with each new Variable that you create from a combination

Keep all terms needed for clarity (usually Plot/SampleID)

Always keep EU as a Variable



Focal: Geno Moderator: Daylength

Structure	Variable	#levels	Block	Experimental Unit
Focal	Geno	4	Dayl, Chamber	Plant
Moderator	Dayl	2	None	Chamber
Combo	Geno:Dayl	8	Chamber	Plant
Design	Chamber	4		
	Plant	16		
	Geno:Chamber	16		
	Geno:Dayl:Chamber	16		
Response	Days	16		

# Analysis

main effects? specific effects? interaction effects?

This is a factorial for Geno x Daylength

Goal: Specific effects and/or Interaction effects

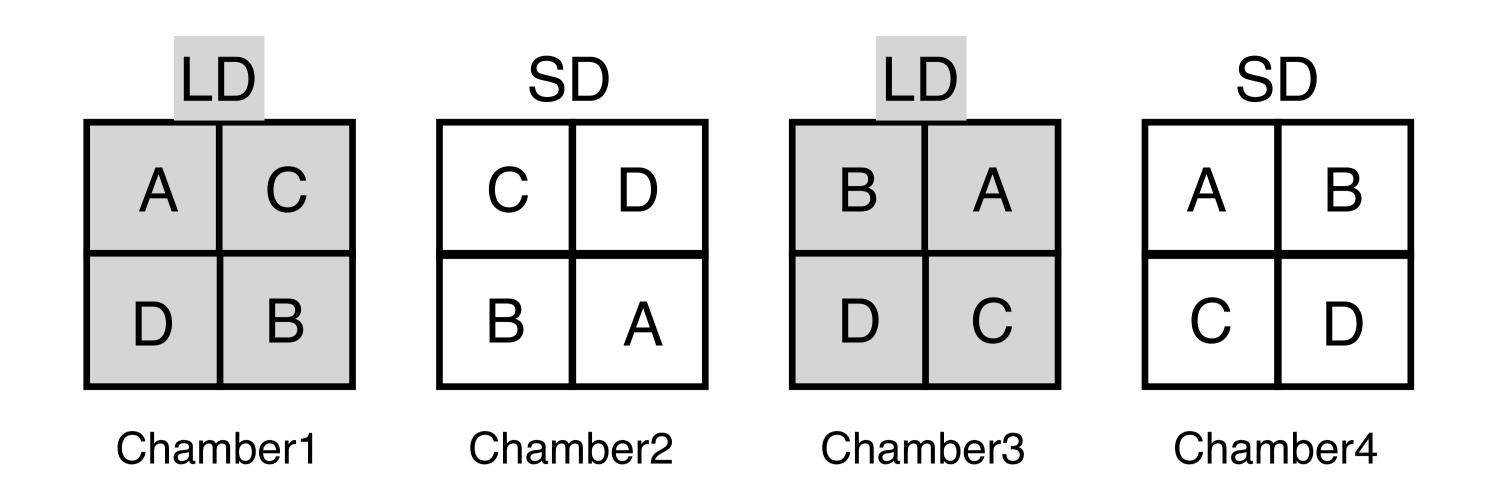
Specific effects of Genotype at each Daylength e.g.  $\delta_{D-A|LD}$ 

Interaction effects of Genotype between Daylengths e.g.  $\delta_{D-A|SD} - \delta_{D-A|LD}$ 

## Strategy:

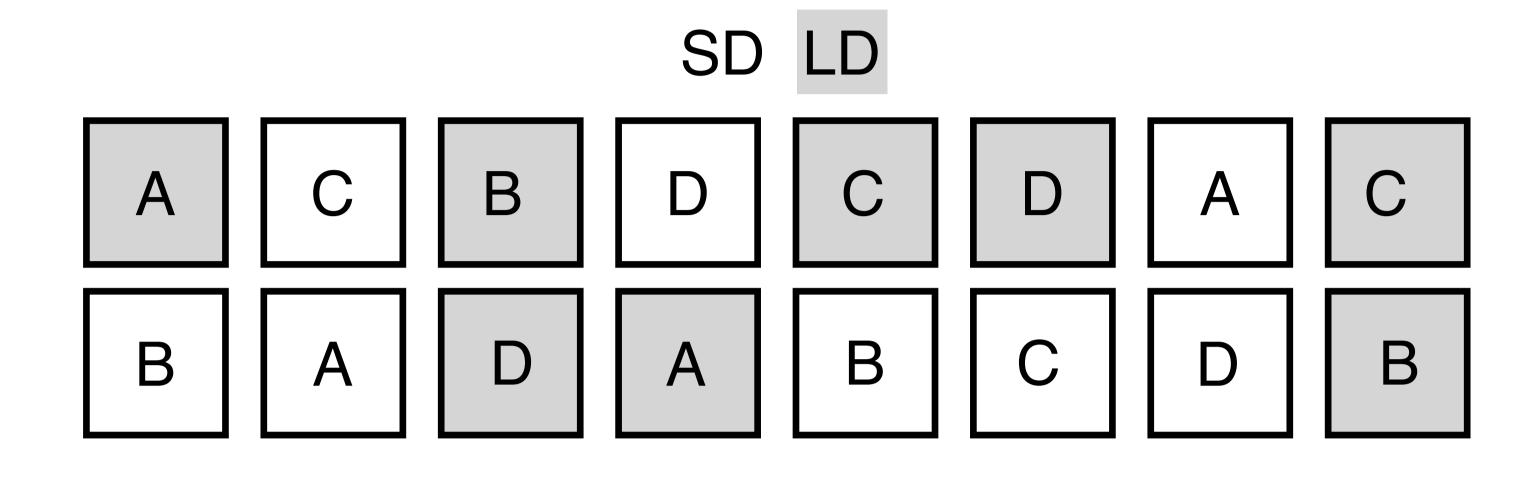
- 1. geno\_means = emmeans(model, specs = 'Genotype', by = 'Daylength')
- 2. geno\_effects = contrast(geno\_means,'pairwise',name = 'Geno\_effects')
- 3. regrouped\_effects = update(geno\_means,by = 'Geno\_effects')
- 4. interaction\_effects = contrast(regrouped\_effects,'pairwise')

What is the consequence of the split-plot design?



Split-Plot Design

2-stage assignment of treatments

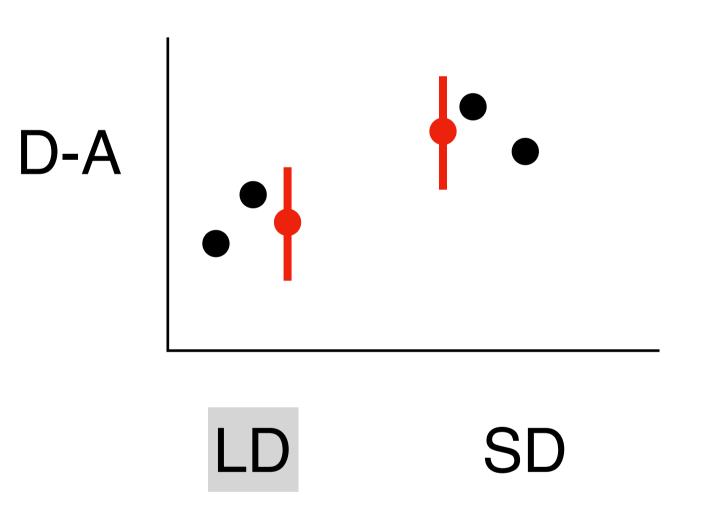


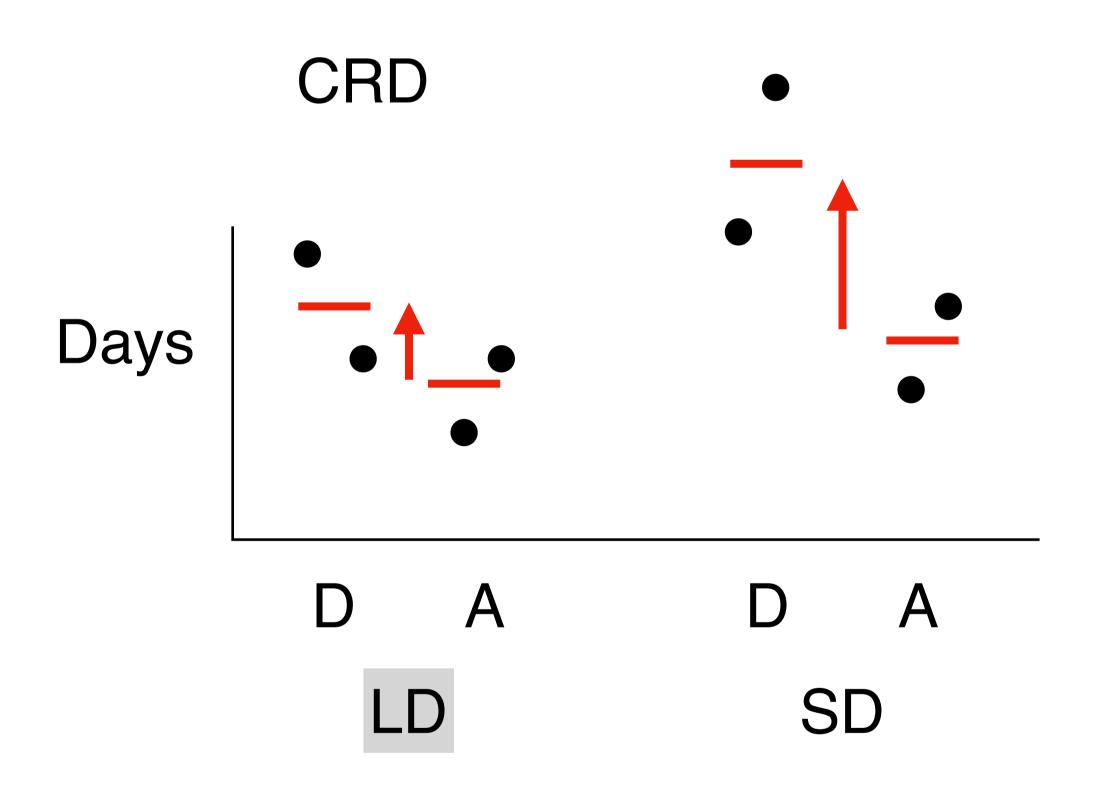
Completely Randomized Design

Assign treatment combos to EUs

# Specific Effects of Genotype at each Daylength

Split-Plot Design





Direct design (with blocks)

$$n_i = 2$$
 per Dayl

Degrees of Freedom:

$$2*(b-1)*(t-1) = 6$$

Indirect design (no blocks)

$$n_i = 2$$
 per Geno:Dayl

No replicates of specific effects

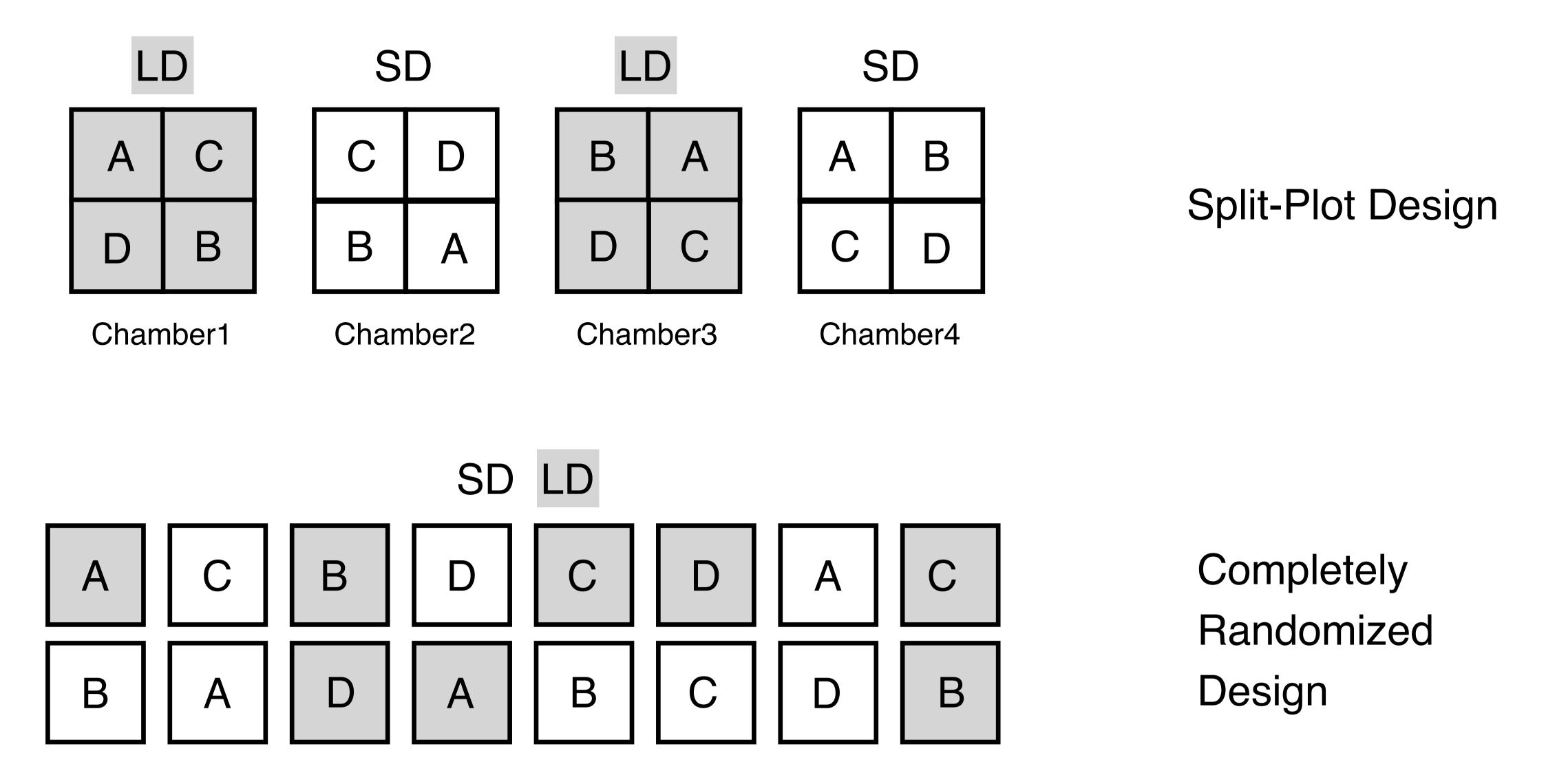
No control for Chamber effects

Degrees of Freedom:

$$2*t*(n_i-1) = 8$$

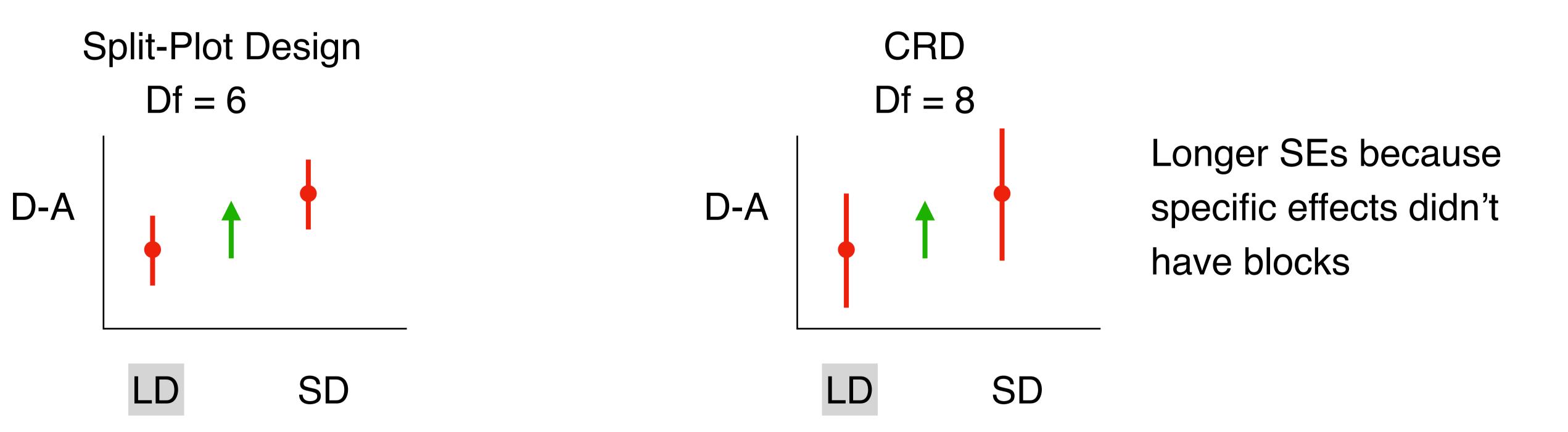
treat each Dayl as a separate expt for Geno

What is the consequence of the split-plot design?



Interaction Effects: Change in Specific effects between Daylengths

e.g. 
$$\delta_{D-A|SD} - \delta_{D-A|LD}$$



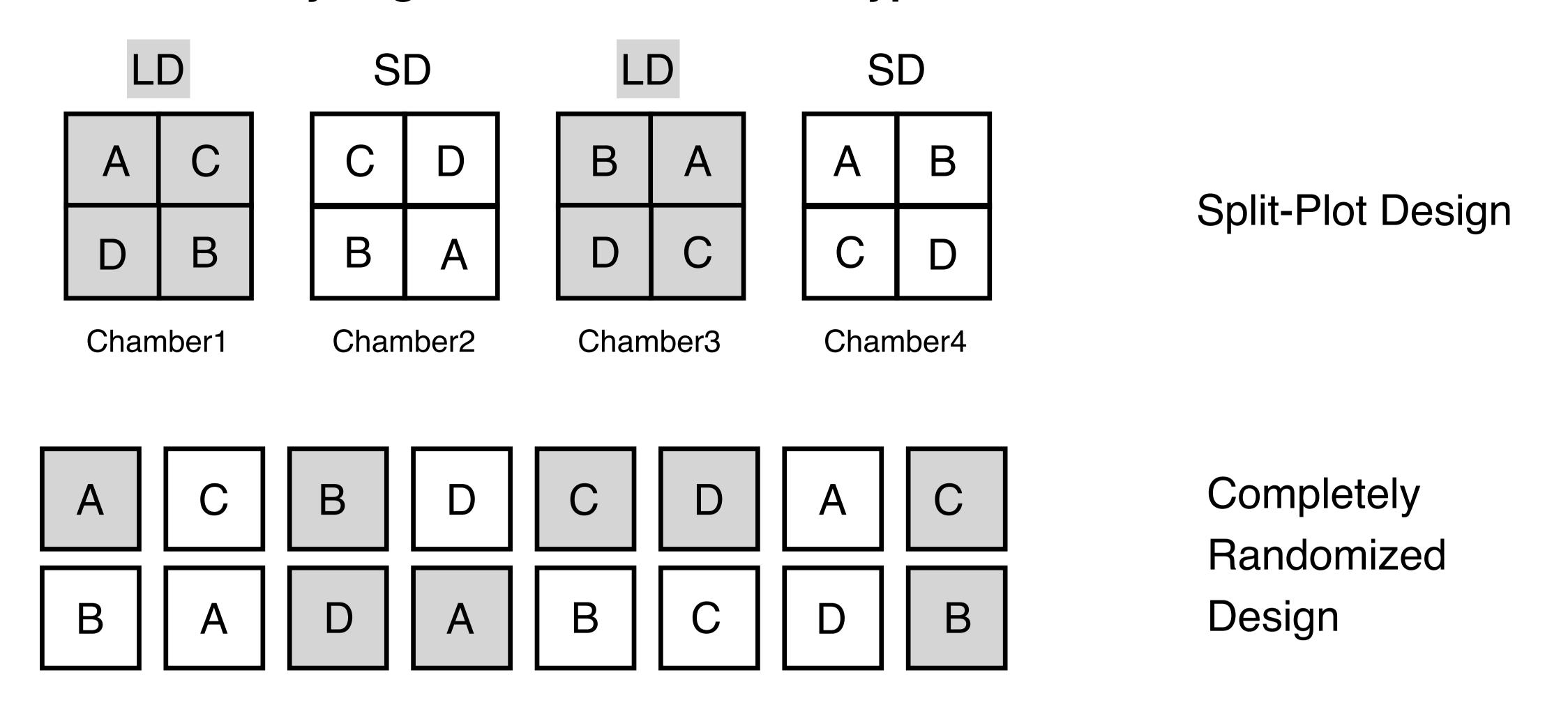
Both are indirect designs for interaction effects

Different chambers are used for LD and SD

No control for chamber effects when comparing between daylengths

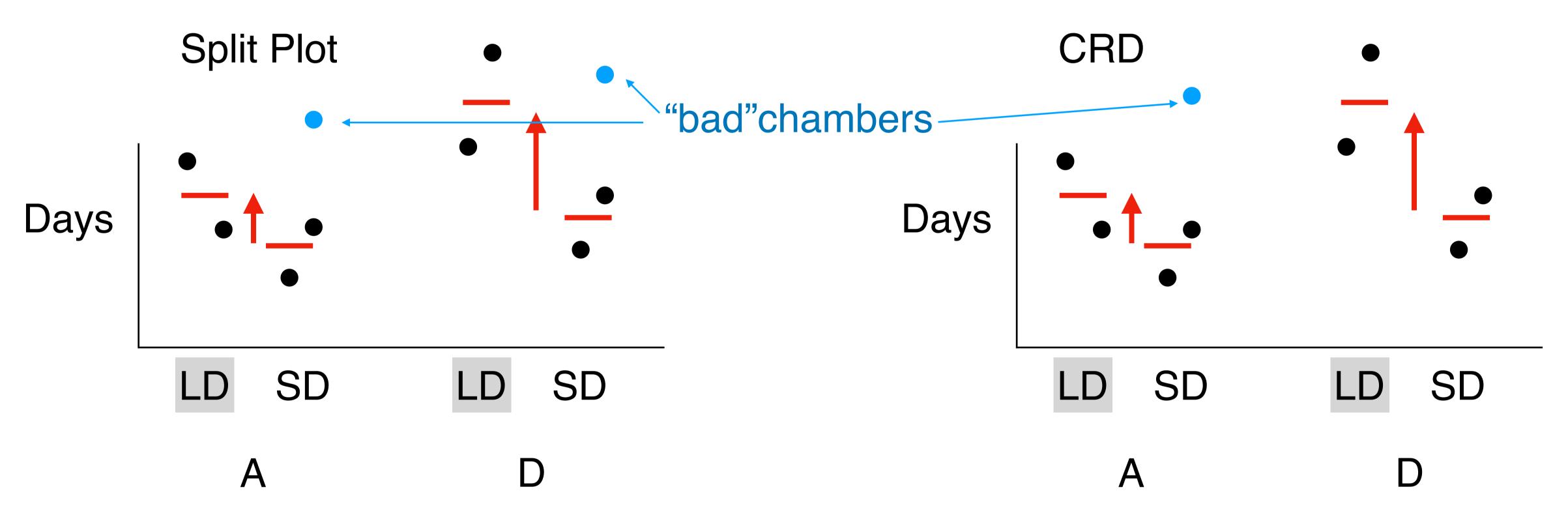
$$\sigma_r(\hat{I}) = \sqrt{2 * \sigma_{effects}^2}$$

Split plot is useful because of chambers (blocks) make specific effects more precise But we don't get replicates of the interactions What about if Daylength was focal and Genotype was moderator?



Specific Effects: Daylength effects for each Genotype

e.g. 
$$\delta_{SD-LD|A}$$



Both are indirect designs for specific effects

All 4 measures of Geno: A are in different chambers

Chambers are not a block for specific effects

Standard Errors are approximately the same in each design

Degrees of Freedom:

each Geno is **not** an independent expt for Geno

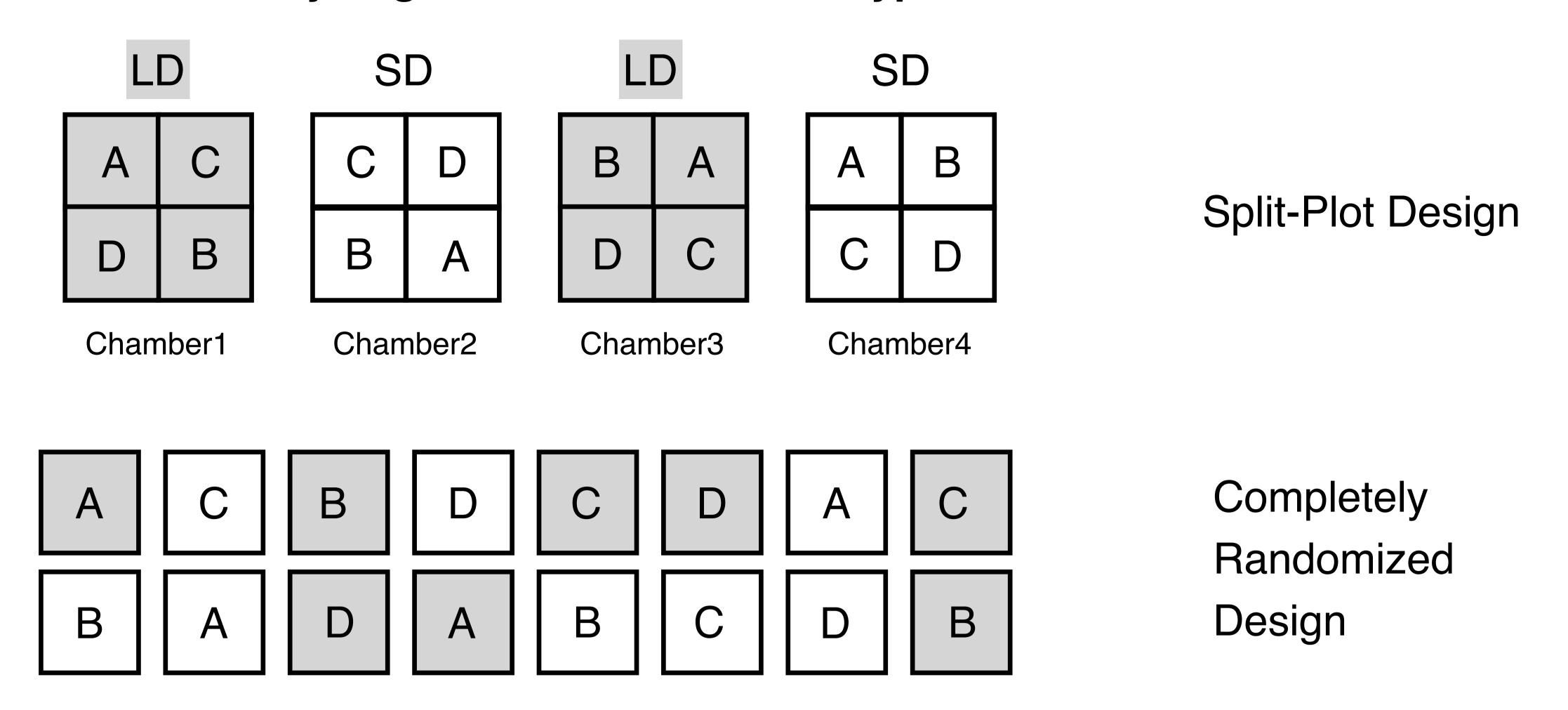
Degrees of Freedom:

$$4*t*(n_{i}-1) = 8$$

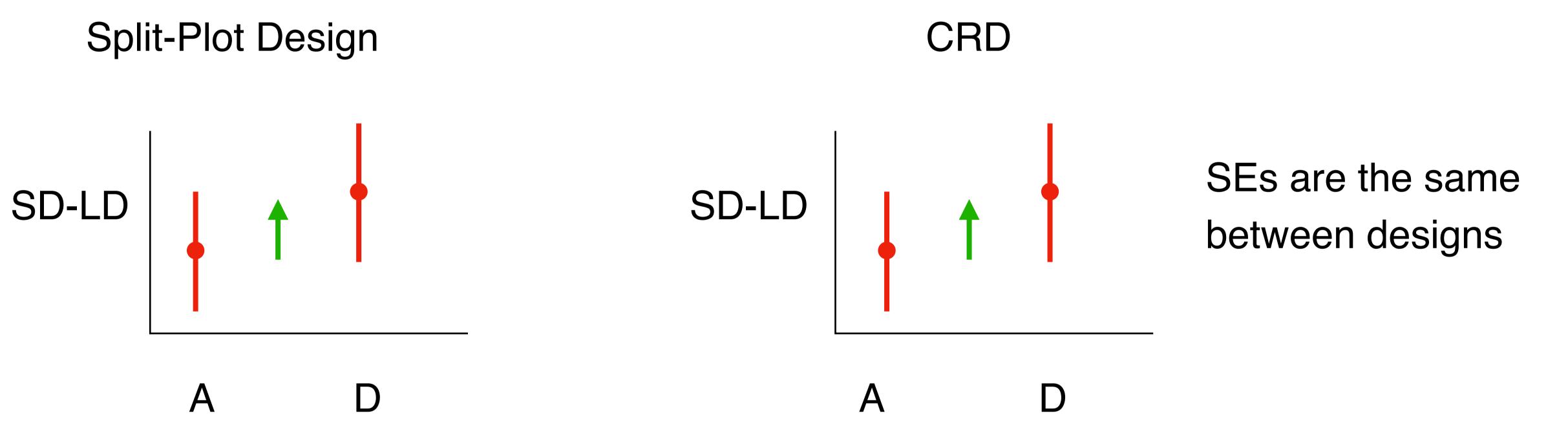
treat each Geno as a separate expt for Dayl

"bad" chambers affect all specific effects in the same way

What about if Daylength was focal and Genotype was moderator?



Interaction Effects: Change in Daylength among Genotypes



Interaction is direct (ish)

SD-LD re-uses chambers for A and D

Interaction is indirect

Each Genotype:Dayl in a different chamber

This controls for chamber variation

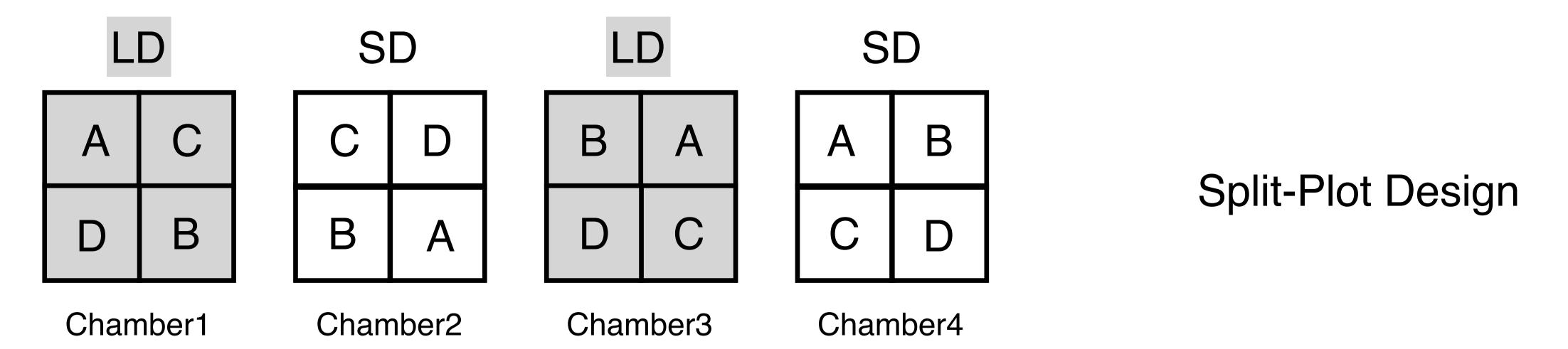
But doesn't allow us multiple replicates of the interaction effect

SEs are the same as when Genotype was focal!

Degrees of freedom are the same as well:

$$2^{*}(b-1)^{*}(t-1) = 6$$
  $4^{*}t^{*}(n_{i}-1) = 8$   $2^{*}t^{*}(n_{i}-1) = 8$ 

### Split plot summary



Factorial design

goal: specific effects and/or interaction effects

Use when blocks are feasible for one treatment but not another

Different treatments have different experimental units

Look at each treatment or treatment:combination separately to identify EUs

Check Blocks carefully, include all necessary Combo terms

Main Plots are always EUs, so are always random!

Different specific effects will have different SEs depending on if the contrast is within or across MainPlots

Genotype specific effects have small SEs

Daylength specific effects have large SEs

Interaction effects have the same SEs

Comparisons among Daylength effects are (sort of) blocked

Degrees of freedom are hard to calculate!

As long as you specify the model table and model formula correctly emmeans will give you correct estimates and SEs

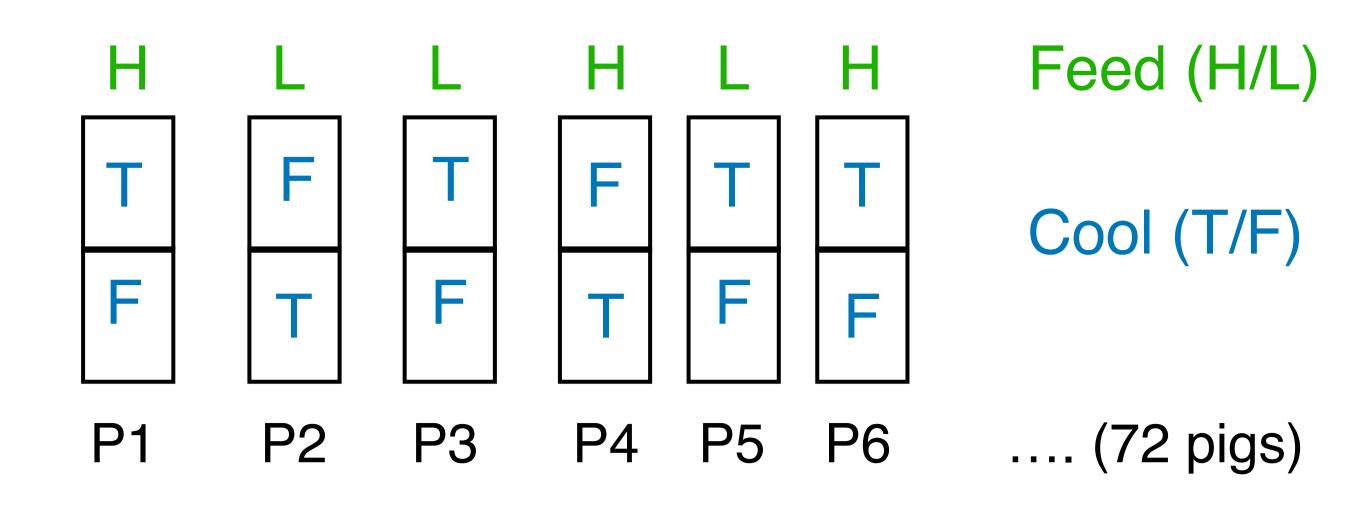
**Goal:** Study the effect of the **cooling process** and **pH** on the tenderness of pork

**Design:** 72 pig carcasses were divided into two groups by pH (low vs high).

Two cuts were made on the right side of each carcass, and each cut was treated with either Tunnel cooling, or Fast (conventional) cooling.

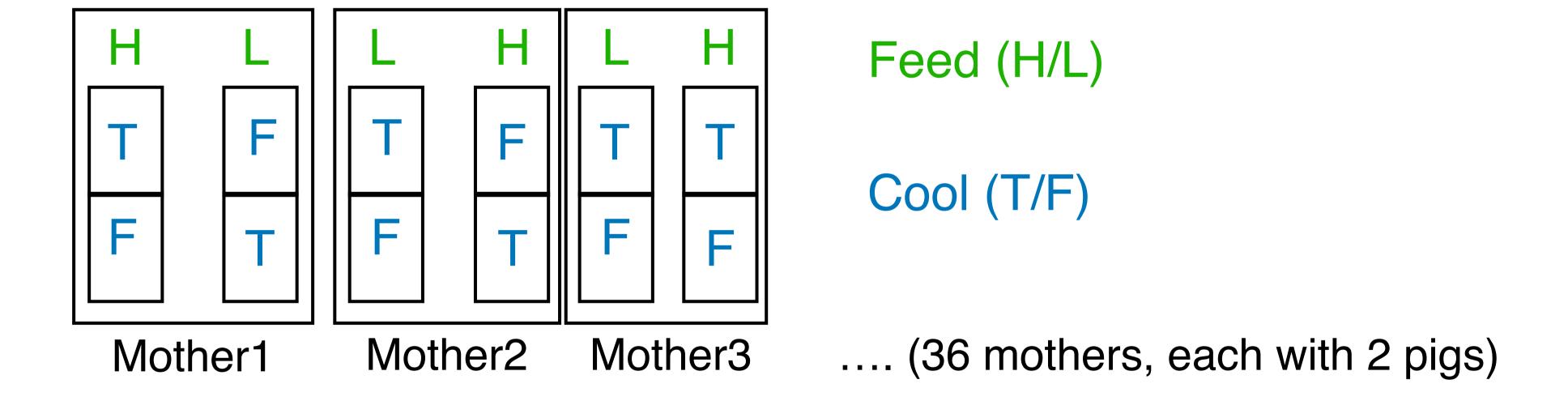
After a storage period the tenderness of each piece was measured

Structure	Variable	#levels	Block	EU	
					What questions should we ask?
					What contrasts should we estimate?



Structure	Variable	#levels	Block	EU
Focal	Feed	2	Cool	Pig
Moderator	Cool	2	Pig	Cut
Combo	Feed:Cool	4	Pig	Cut
Design	Pig	72		
	Cut	144		
	Cool:Pig	144		
	Feed:Cool:Pig	144		
Response	Tenderness	144		

Imer(Tenderness~Feed+Cool+Feed:Cool+(1|Pig))



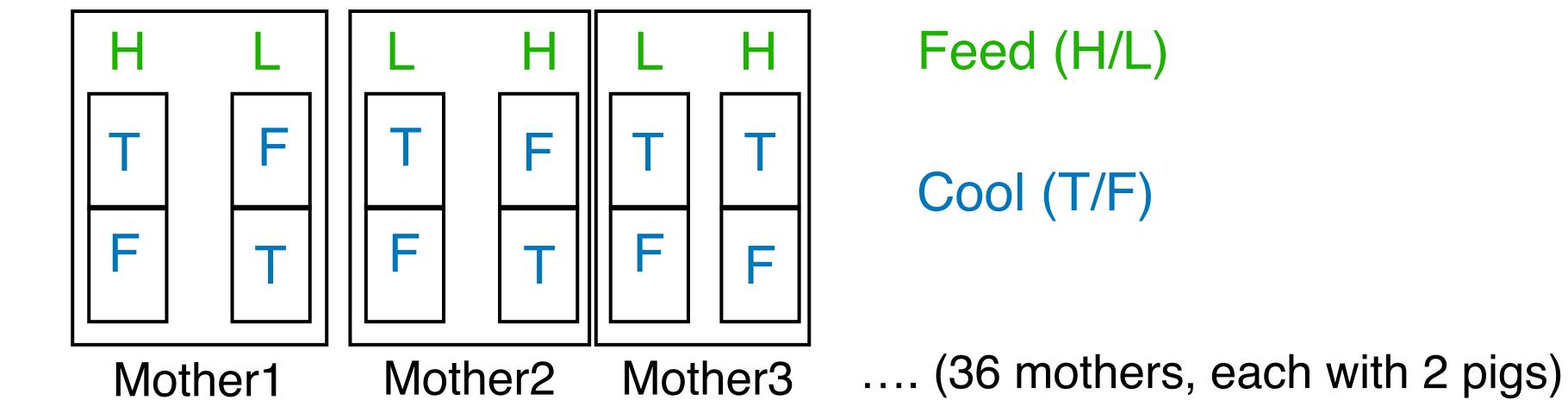
What questions should we ask?

What contrasts should we estimate?

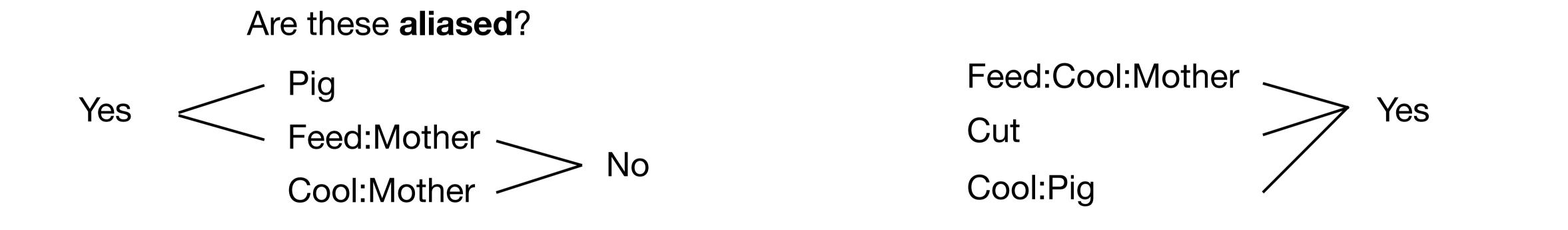
Mother = Block for both Feed and Cool

RCBD for Feed

RCBD with reps for Cool (so will include Cool:Mother as EU)



Structure	Variable	#levels	Block	EU
Focal	Feed	2	Cool, Mother	Pig
Moderator	Cool	2	Mother, Pig	Cut
Combo	Feed:Cool	4	Mother, Pig	Cut
Design	Mother	36		
	Pig	72		
	Feed:Mother	72		
	Cool:Mother	72		
	Feed:Cool:Mother	144		
	Cut	144		
	Cool:Pig	144		
Response	Tenderness	144		



Imer(Tenderness ~ Feed + Cool + Feed:Cool + Mother + (1:Cool:Mother) + (1|Pig))

Mother is a complete block for all treatments

It doesn't matter if it is declared random

Feed:Mother is aliased with Pig. Since Pig is an EU, it must be random

You can choose either term

Cool:Mother is random if we want **main effects** of Cool or Feed:Cool averaged over mothers.

We have 36 mothers, so with only 2 reps of each cool level per mother, reporting specific effects per mother would not be very powerful