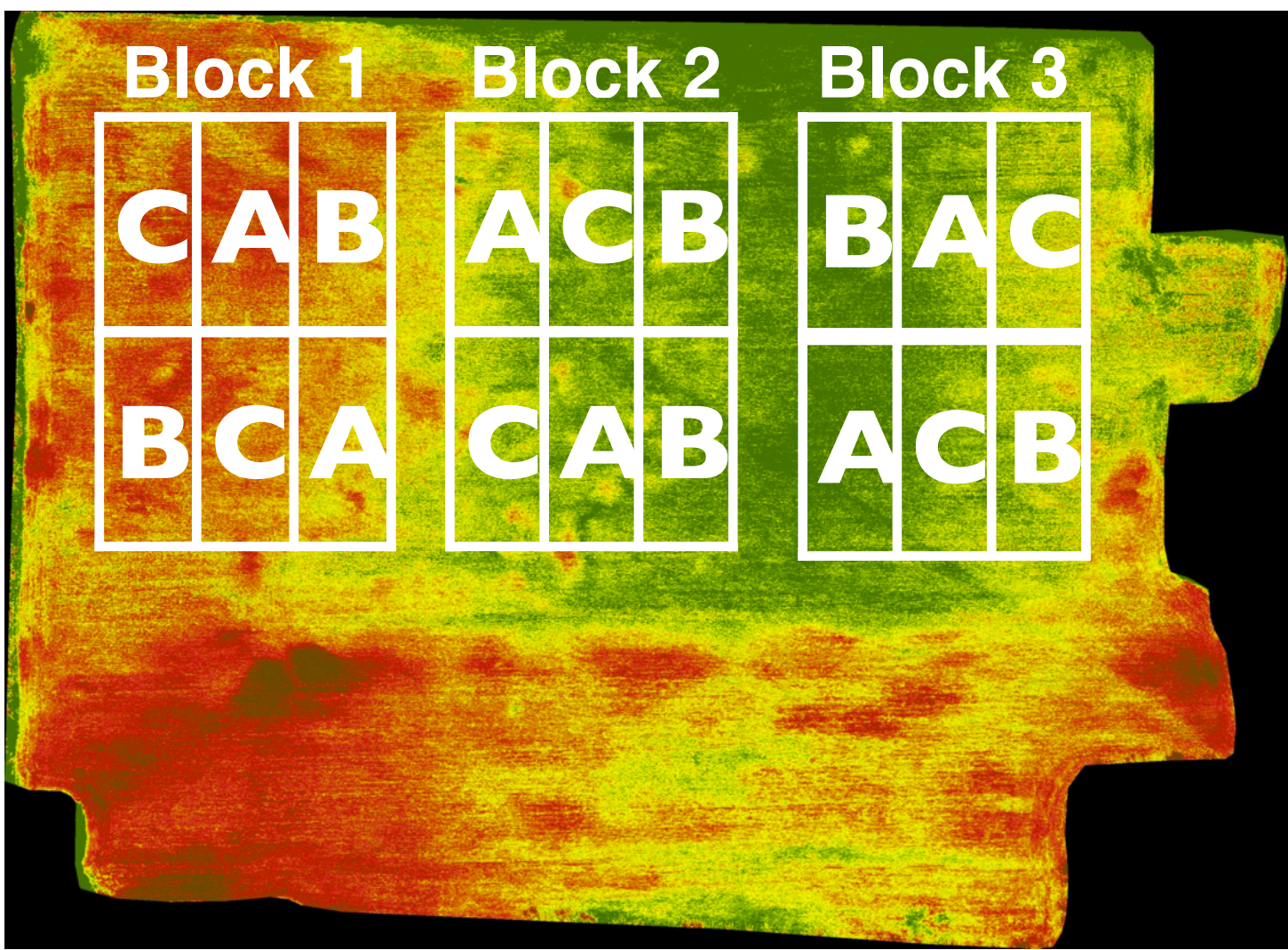


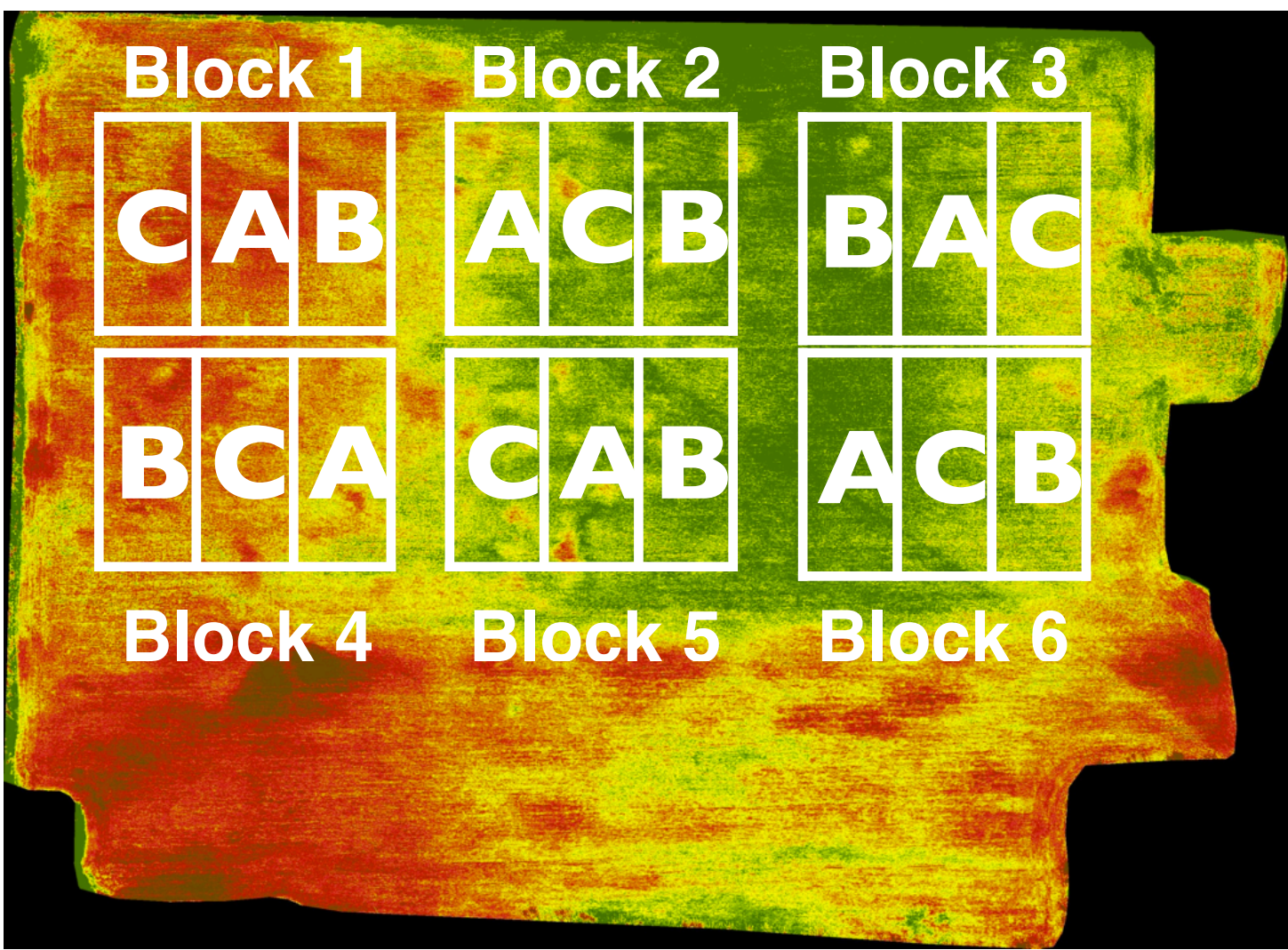
Optimal number of blocks



No Blocks



3 Blocks



6 Blocks

Compare similar EU s^2_{pooled} or s^2_{error}
Worst \longrightarrow Best

Degrees of Freedom - average effect (main effect)
 $k \cdot (n_i - 1) = 15$ $(k-1) \cdot (b-1) = 4$ $(k-1) \cdot (b - 1) = 10$

Degrees of Freedom - specific effects
0 $b \cdot k \cdot (n_{ij}-1) = 9$ 0

Variability of treatment effects across the field

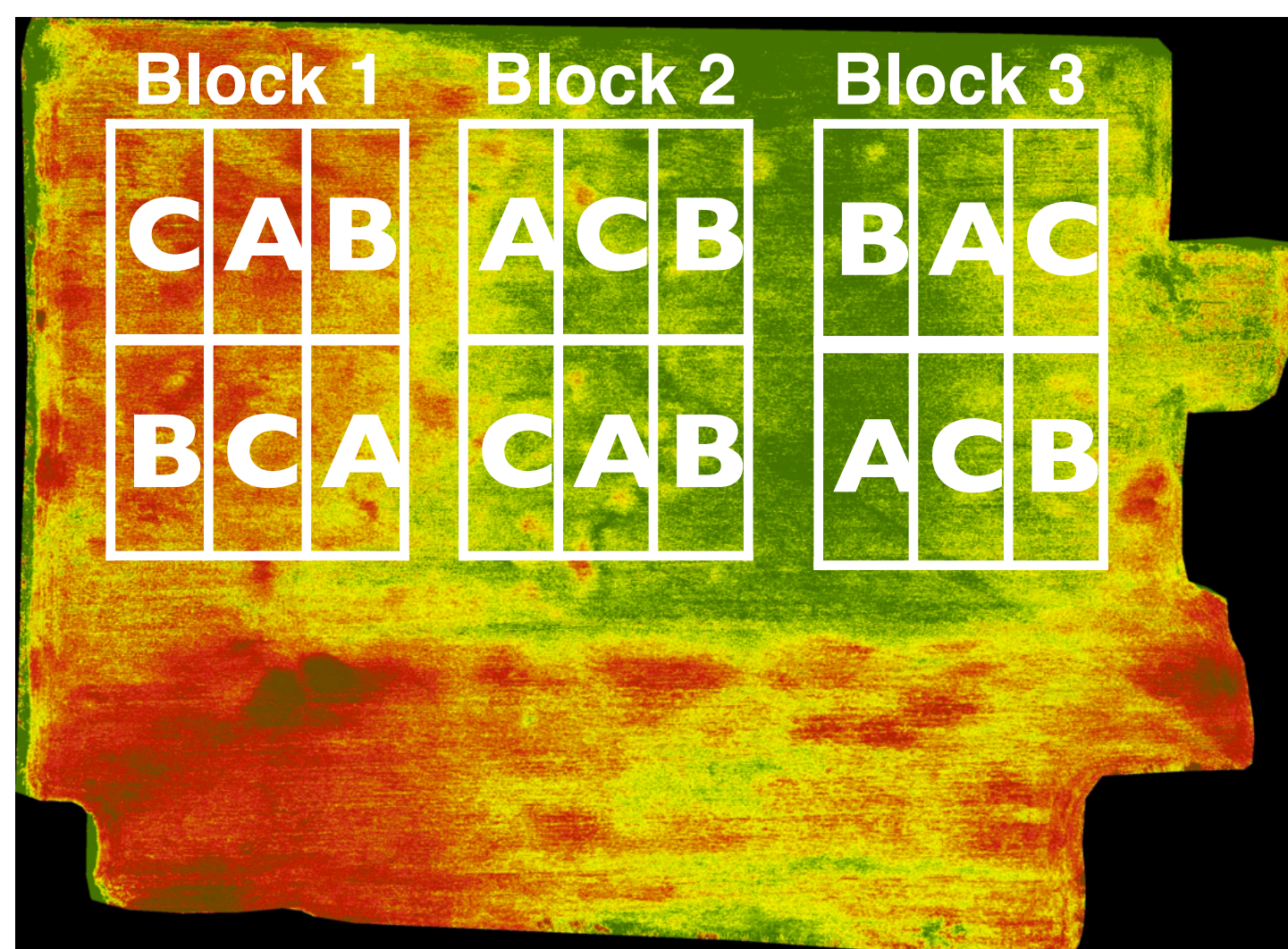
Violates assumptions Test Treatment:Block interactions Increases s^2 , SED
Detect through diagnostics

- More replicates per block means:
- More confidence in Treatment:Block interactions
 - Less power for main effects
 - Higher DF
 - Less similar EU (higher s^2)

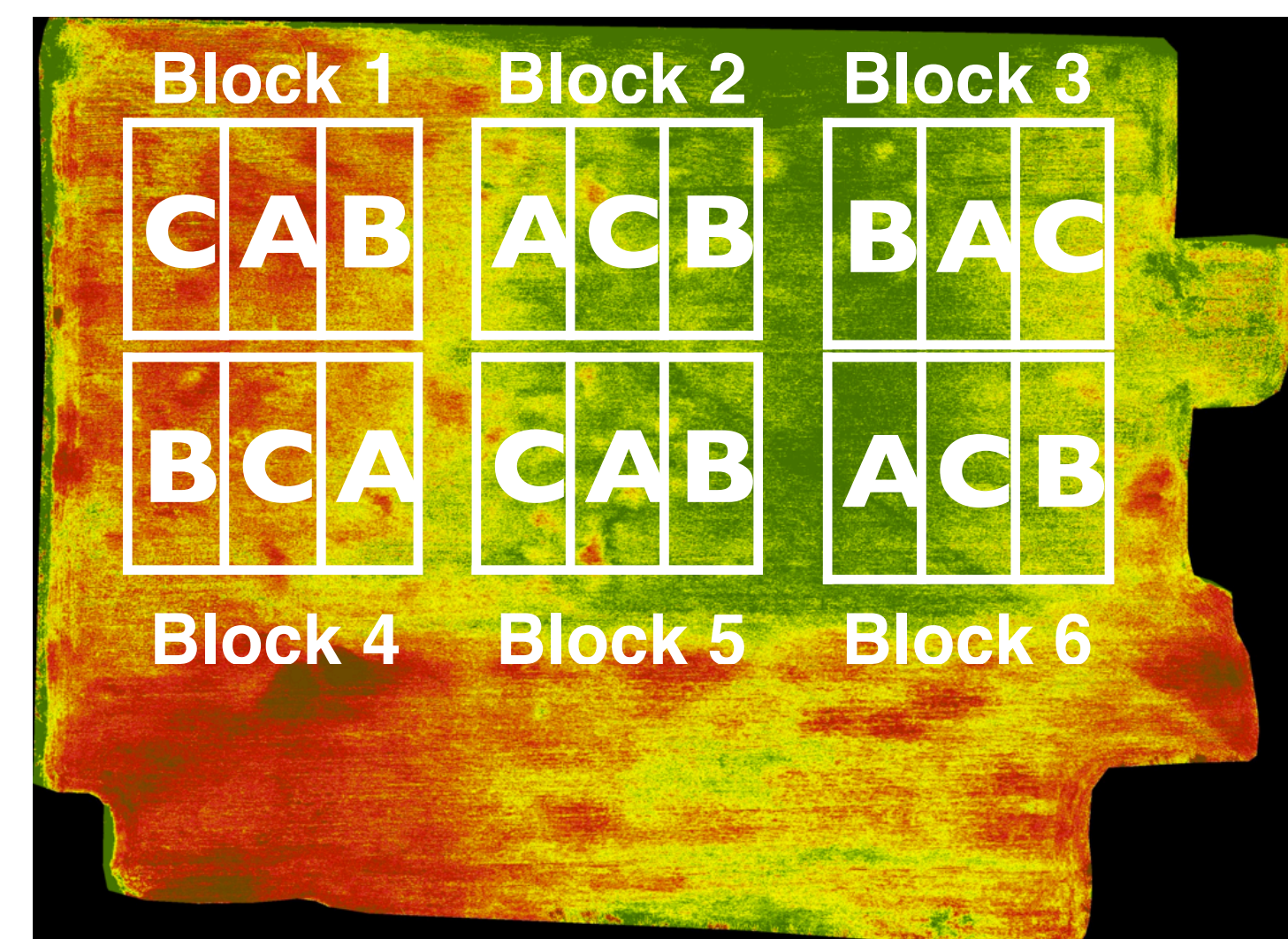
Optimal number of blocks



No Blocks



3 Blocks



6 Blocks

Recommendations:

Don't block unless you can identify **clusters** of EU

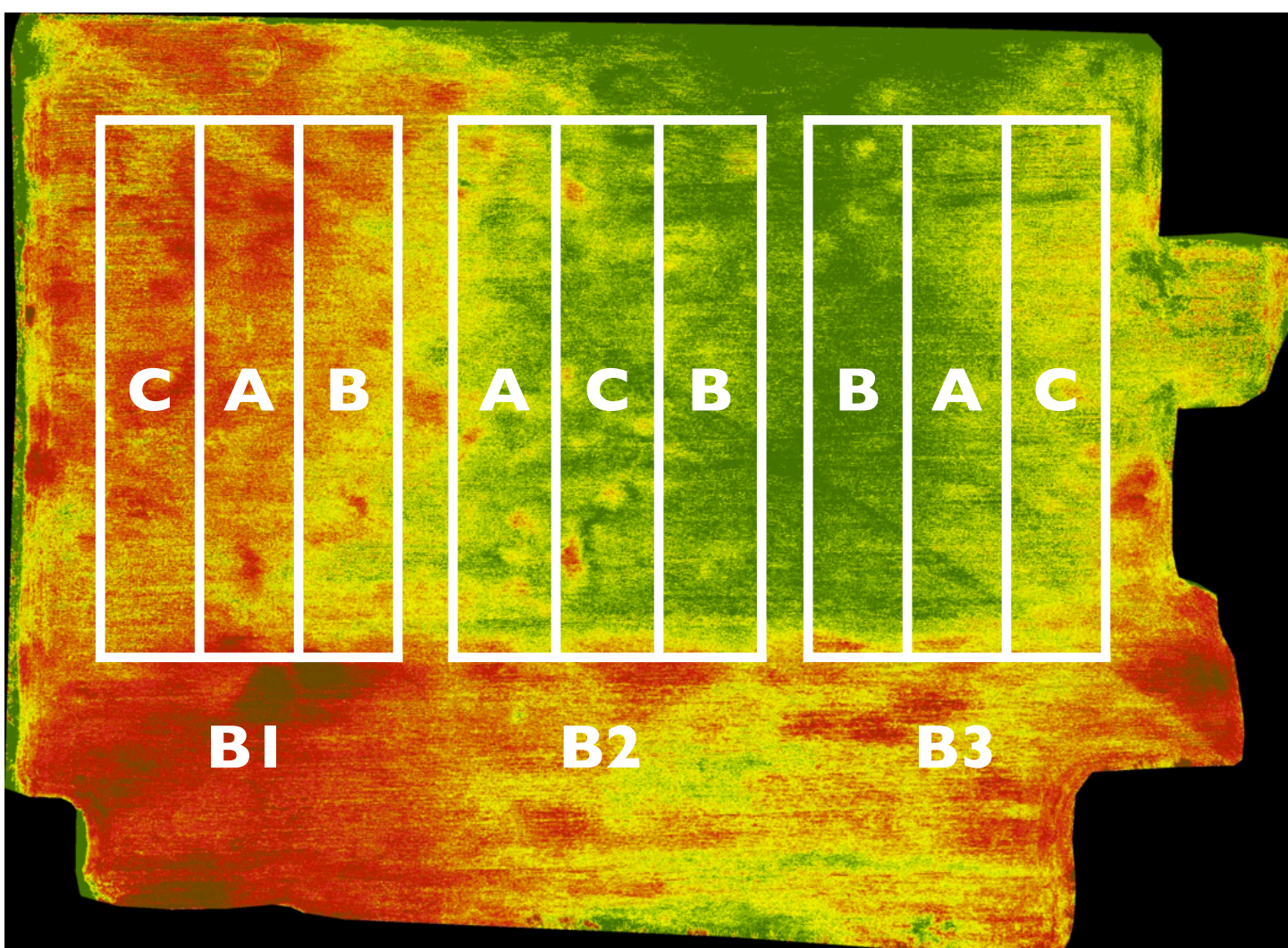
More (small) blocks are best for studying **main effects**

But, blocks should be representative of your target population

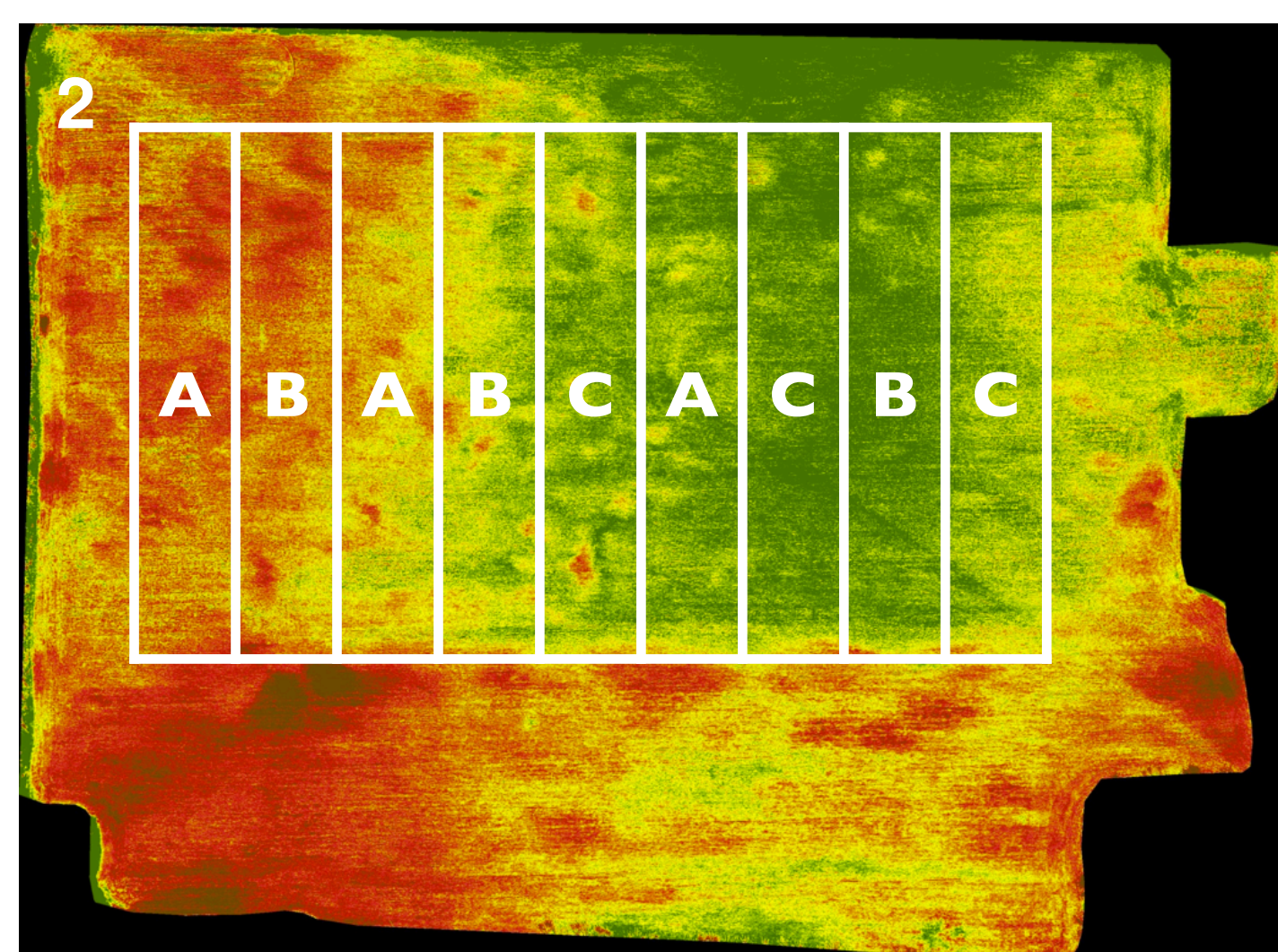
Only do replication of treatments within blocks if:

- 1) You want to know if treatment effects vary in your population
- 2) It's not feasible to do smaller blocks that “make sense”

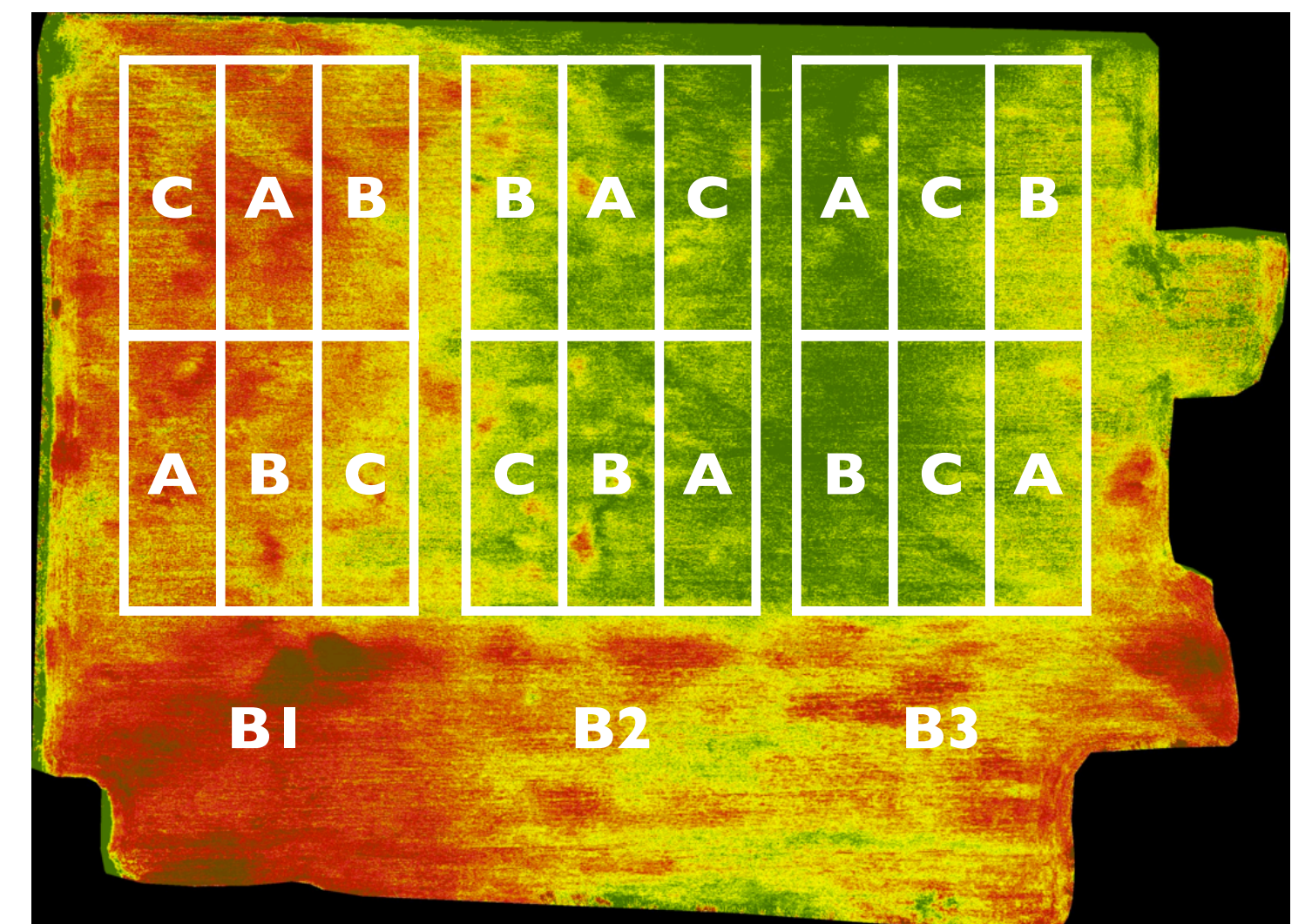
Recommending Designs



RCBD



CRD



RCBD with Reps

Which design would you use to make an Insecticide recommendation to **this farmer** (in this field)?

Reps are overkill, replicate plots within blocks are sub-samples; not-interspersed

Say the farmer could target regions within a field? Which would you use?

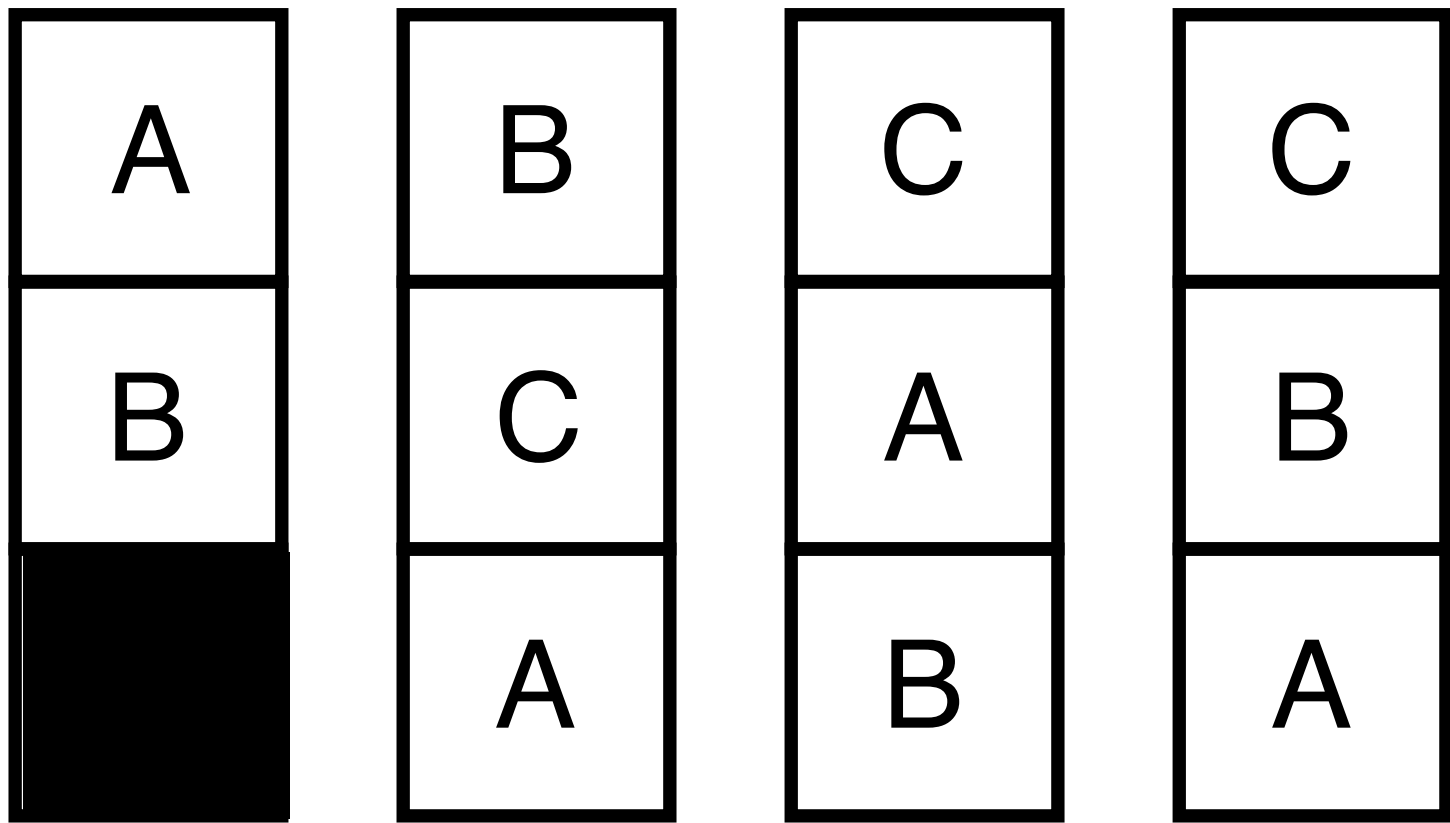
RCBD with reps is necessary (more like a factorial)

What design would you use to make a recommendation in a new field?

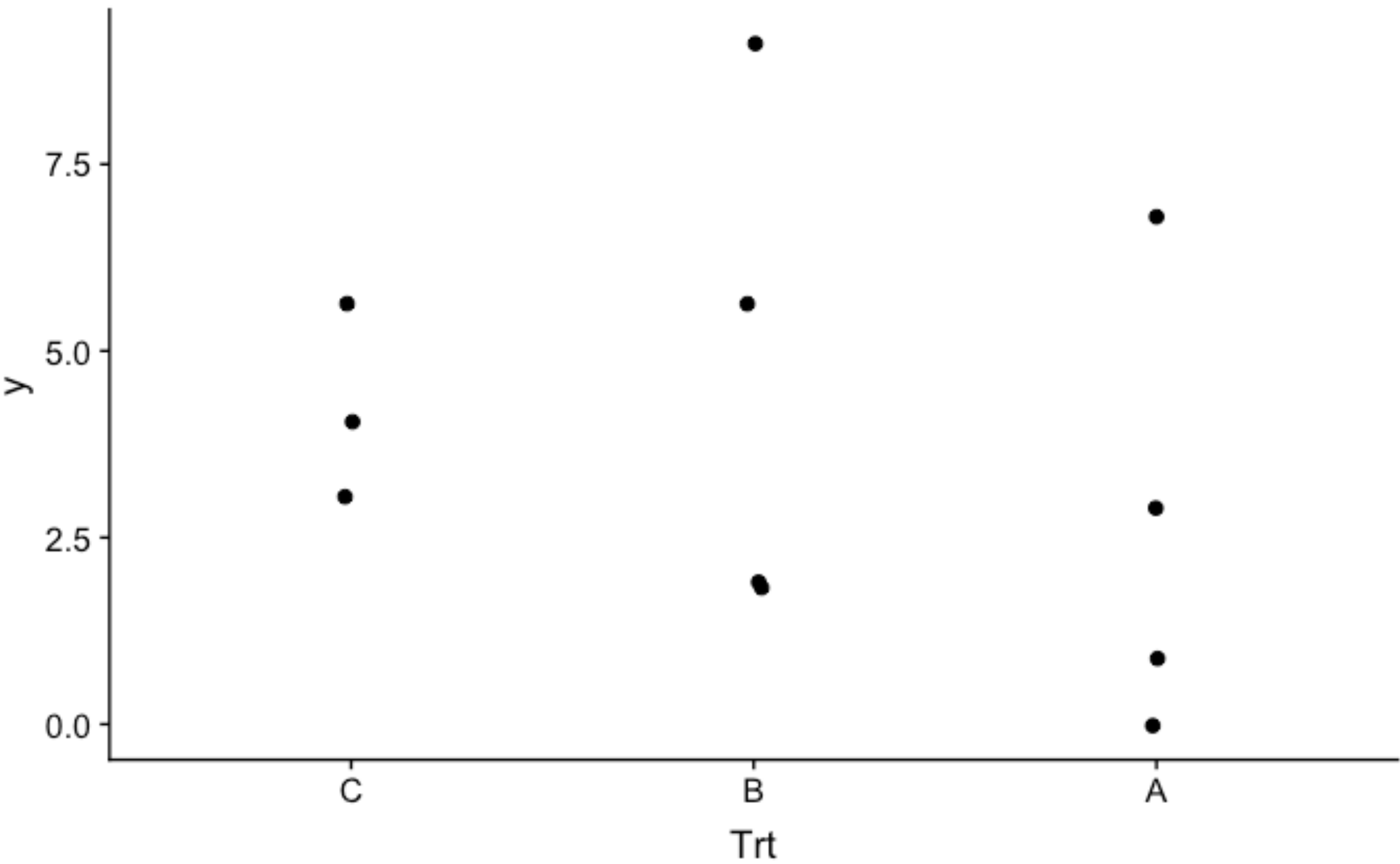
RCBD with Fields as blocks to estimate **main effect** of insecticide across all fields

Factorial with **Field types** as moderator to estimate **specific effects** in **certain types of fields**

This would be an example of a **split-plot experiment**



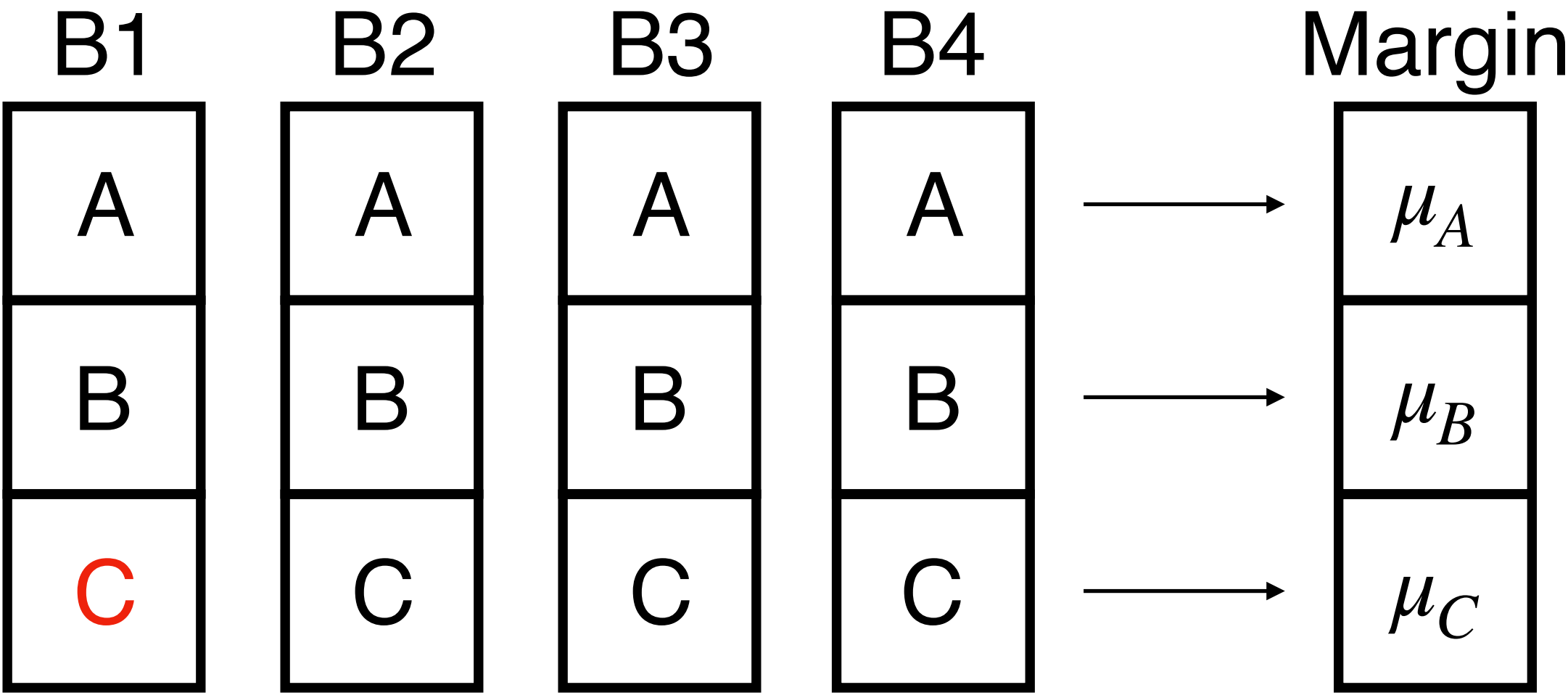
	μ_A	μ_B	μ_C
#reps	4	4	3
estimate	$\frac{\sum y_{Ai}}{4}$	$\frac{\sum y_{Bi}}{4}$	$\frac{\sum y_{Ci}}{3}$
$\sigma_{\hat{\mu}}^2$	$\frac{\sigma_{EU}^2}{4}$	$\frac{\sigma_{EU}^2}{4}$	$\frac{\sigma_{EU}^2}{3}$



```
```{r}
aggregate(y~Trt, data_m, FUN=mean)
```
```

| Trt
<fctr> | y
<dbl> |
|---------------|------------|
| C | 4.333333 |
| B | 4.750000 |
| A | 2.750000 |

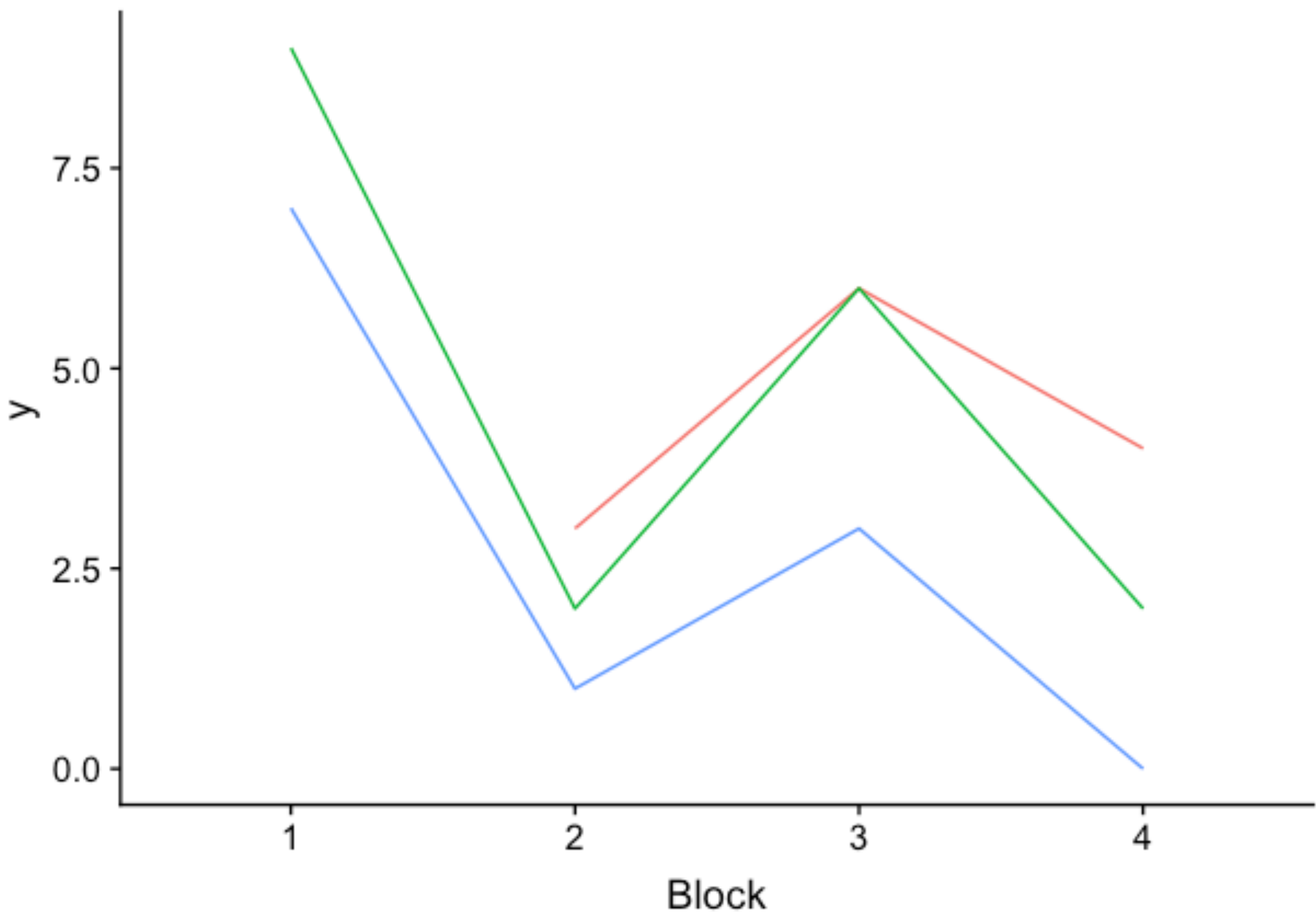
3 rows



“emmeans” = Expected Marginal Means

predict the mean had the experiment been complete

SEs and df adjusted for actual data



Trt

— C

— B

— A

```
library(emmeans)
emmeans(lm(y~Block+Trt,data_m),~Trt)
```

| Trt | emmean | SE | df | lower.CL | upper.CL |
|-----|--------|-----------|----|----------|----------|
| C | 5.75 | 0.3872983 | 5 | 4.754418 | 6.745582 |
| B | 4.75 | 0.3162278 | 5 | 3.937111 | 5.562889 |
| A | 2.75 | 0.3162278 | 5 | 1.937111 | 3.562889 |

Results are averaged over the levels of: Block

Confidence level used: 0.95

$\hat{\delta}_{B-A}$ = ave (B-A) in 4 blocks

$\hat{\delta}_{C-A}$ = ave (C-A) in 3 blocks

= (C-B) - (B-A)

B2-B4 B1

low SE (within blocks)

high SE (across blocks)

A1 is **connected** to C(2-4) through B

Analysis with missing cells

emmeans: same as normal

ANOVA: order of terms in formula matters

```
library(emmeans)
lm1 = lm(y~Block+Trt,data_m)
anova(lm1)
```

Analysis of Variance Table

Response: y

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|-----------|----|--------|---------|---------|---------------|
| Block | 3 | 58.909 | 19.636 | 49.091 | 0.0003933 *** |
| Trt | 2 | 16.000 | 8.000 | 20.000 | 0.0041152 ** |
| Residuals | 5 | 2.000 | 0.400 | | |

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

```
library(emmeans)
lm_switch = lm(y~Trt+Block,data_m)
anova(lm_switch)
```

Analysis of Variance Table

Response: y

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|-----------|----|--------|---------|---------|---------------|
| Trt | 2 | 8.742 | 4.3712 | 10.928 | 0.0149561 * |
| Block | 3 | 66.167 | 22.0556 | 55.139 | 0.0002972 *** |
| Residuals | 5 | 2.000 | 0.4000 | | |

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

*put blocks first

Can declare Blocks as random, may reduce SE of differences if there are many blocks

y ~ (1|Block) + Trt

Say we want to evaluate three types of eyeglass materials

Can we use blocks in this experiment?

 can be a block, but cannot be a **complete block**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| A | B | C | C | A | B | | |
| B | C | A | B | C | A | | |
| C | A | B | A | B | C | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| A | 1 | | 1 | | 1 | 1 |
| B | 1 | 1 | | 1 | | 1 |
| C | | 1 | 1 | 1 | 1 | |

Balanced Incomplete Block Design (BIBD)

Incomplete Block: not every treatment in every block

t: # treatments k: # treatments/block $k < t$

Balanced: every pair occurs together same # of times

λ : ave # times pairs occur together $\lambda = 2$

smaller blocks than RCBD

more homogeneous, easier to run

all pairwise comparisons equally powerful

optimized for “pairwise”

hard with many treatments

needs to be very large to achieve balance

$t=8, k=4 \Rightarrow$ need at least 14 blocks

Description:

b: # blocks

t: # treatments

k: # trt/block

r: # reps/trt

λ : ave # times pairs
occur together

$\lambda = r(k-1)/(t-1)$

Augmented design

Incomplete block design

Control treatments

New treatments

optimized for $\hat{\delta}_{\text{ctrl-new}}$

Dunnett-type

| B1 | B2 | B3 |
|----|----|----|
| A | C | C |
| B | A | C |
| C | B | A |
| 1 | 9 | 17 |
| 2 | 10 | 18 |
| 3 | 11 | 19 |
| 4 | 12 | 20 |
| 5 | 13 | 21 |
| 6 | 14 | 22 |
| 7 | 15 | 23 |
| 8 | 16 | 24 |

RCBD for the controls

Un-replicated for new treatments

But all connected

$(11-1) = (11-A) - (A-1)$

$\hat{\delta}_{B-A}$

$\hat{\delta}_{2-A}$ Different SEs

$\hat{\delta}_{12-2}$

| | | | |
|---|---|---|---|
| A | B | C | D |
| C | D | A | B |

Unconnected design

```
```{r}
table(data$Block,data$Trt)|
```
```

| | | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| 1 | 1 | 1 | 0 | 1 | 0 |
| 2 | 0 | 1 | 0 | 1 | |
| 3 | 1 | 0 | 1 | 0 | |
| 4 | 0 | 1 | 0 | 1 | |

```
```{r}
lm1 = lm(y~Block+Trt,data)
emmeans(lm1,pairwise~Trt)$contrasts
```
```

| contrast | estimate | SE | df | t.ratio | p.value |
|----------|------------|-----------|----|---------|---------|
| 1 - 2 | nonEst | NA | NA | NA | NA |
| 1 - 3 | -0.4306599 | 0.4025535 | 2 | -1.070 | 0.7381 |
| 1 - 4 | nonEst | NA | NA | NA | NA |
| 2 - 3 | nonEst | NA | NA | NA | NA |
| 2 - 4 | 0.2626295 | 0.4025535 | 2 | 0.652 | 0.9068 |
| 3 - 4 | nonEst | NA | NA | NA | NA |

Results are averaged over the levels of: Block
P value adjustment: tukey method for comparing a family of 4 estimates

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

| Rep 1 | Rep 2 | Rep 3 | Rep 4 |
|-------|-------|-------|-------|
| 1 3 2 | 8 5 2 | 3 8 4 | 2 4 9 |
| 6 4 5 | 1 7 4 | 7 6 2 | 8 6 1 |
| 7 8 9 | 6 9 3 | 5 9 1 | 7 5 3 |

Blocks?

Incomplete?

Balanced?

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

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| <table><tr><td>A</td><td>C</td></tr><tr><td>D</td><td>B</td></tr></table> <div>Chamber1</div> | A | C | D | B | <table><tr><td>C</td><td>D</td></tr><tr><td>B</td><td>A</td></tr></table> <div>Chamber2</div> | C | D | B | A | <table><tr><td>B</td><td>A</td></tr><tr><td>D</td><td>C</td></tr></table> <div>Chamber3</div> | B | A | D | C | <table><tr><td>A</td><td>B</td></tr><tr><td>C</td><td>D</td></tr></table> <div>Chamber4</div> | A | B | C | D |
| A | C | | | | | | | | | | | | | | | | | | |
| D | B | | | | | | | | | | | | | | | | | | |
| C | D | | | | | | | | | | | | | | | | | | |
| B | A | | | | | | | | | | | | | | | | | | |
| B | A | | | | | | | | | | | | | | | | | | |
| D | C | | | | | | | | | | | | | | | | | | |
| A | B | | | | | | | | | | | | | | | | | | |
| C | D | | | | | | | | | | | | | | | | | | |

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 effects among genotypes

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

| | | | | | | | | | | | | | | | | | | | |
|---|----------|----------|----------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LD | SD | LD | SD | | | | | | | | | | | | | | | | |
| <table><tr><td>A</td><td>C</td></tr><tr><td>D</td><td>B</td></tr></table> | A | C | D | B | <table><tr><td>C</td><td>D</td></tr><tr><td>B</td><td>A</td></tr></table> | C | D | B | A | <table><tr><td>B</td><td>A</td></tr><tr><td>D</td><td>C</td></tr></table> | B | A | D | C | <table><tr><td>A</td><td>B</td></tr><tr><td>C</td><td>D</td></tr></table> | A | B | C | D |
| A | C | | | | | | | | | | | | | | | | | | |
| D | B | | | | | | | | | | | | | | | | | | |
| C | D | | | | | | | | | | | | | | | | | | |
| B | A | | | | | | | | | | | | | | | | | | |
| B | A | | | | | | | | | | | | | | | | | | |
| D | C | | | | | | | | | | | | | | | | | | |
| A | B | | | | | | | | | | | | | | | | | | |
| C | D | | | | | | | | | | | | | | | | | | |
| Chamber1 | Chamber2 | Chamber3 | Chamber4 | | | | | | | | | | | | | | | | |

What type of experiment is this? Factorial? RCBD? Split-plot factorial

What is the EU? Depends on the treatment

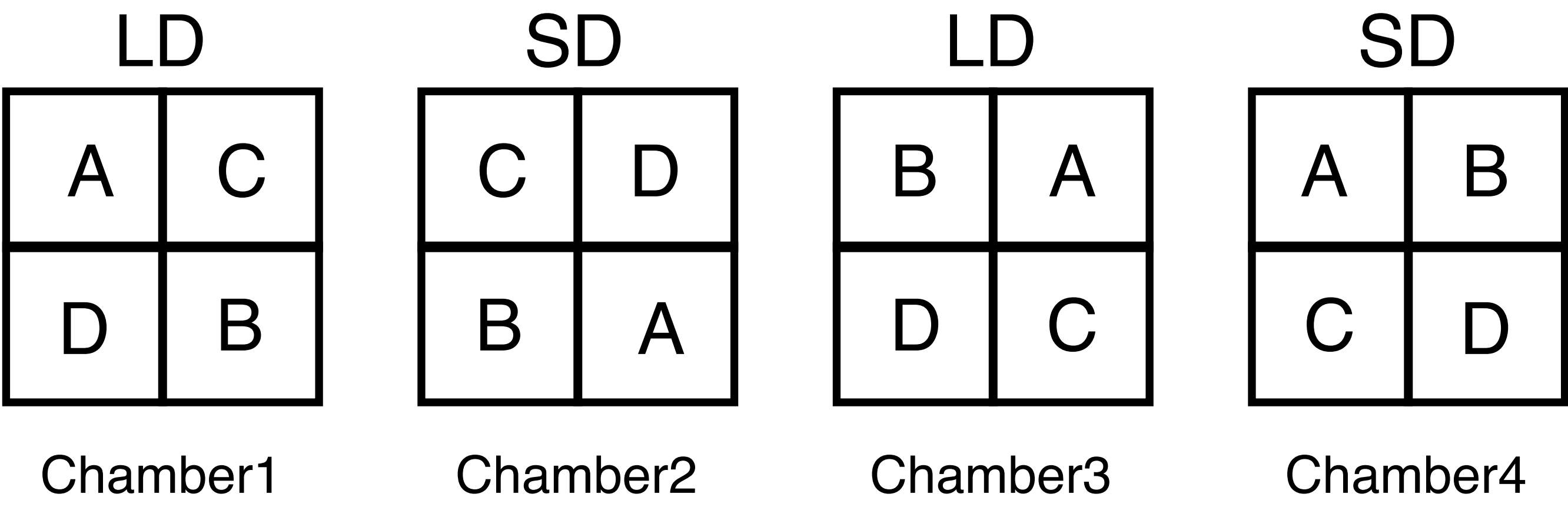
What estimates does the researcher want to report? focal = Genotype moderator = Daylength

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

Sacrifice replication of MainPlot treatment

Two-stage randomization

to increase replication of SubPlot treatment

- 1) Daylength treatments randomized to chambers
- 2) Genotypes randomized to pots **within** each chamber

and treatment combinations

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

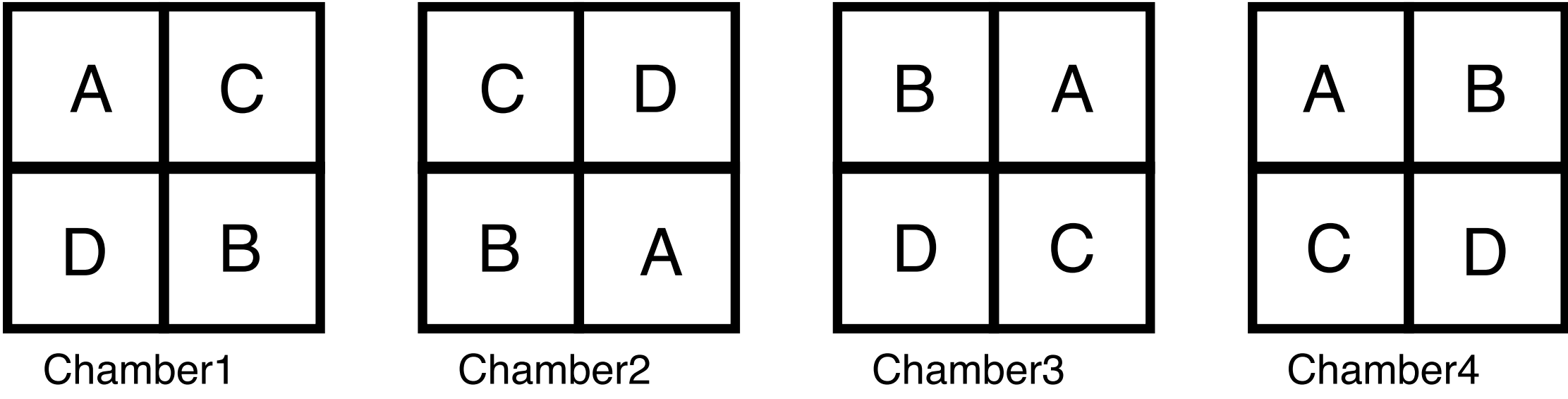
Be sure to declare them all!

Three experiments

EU?

Block?

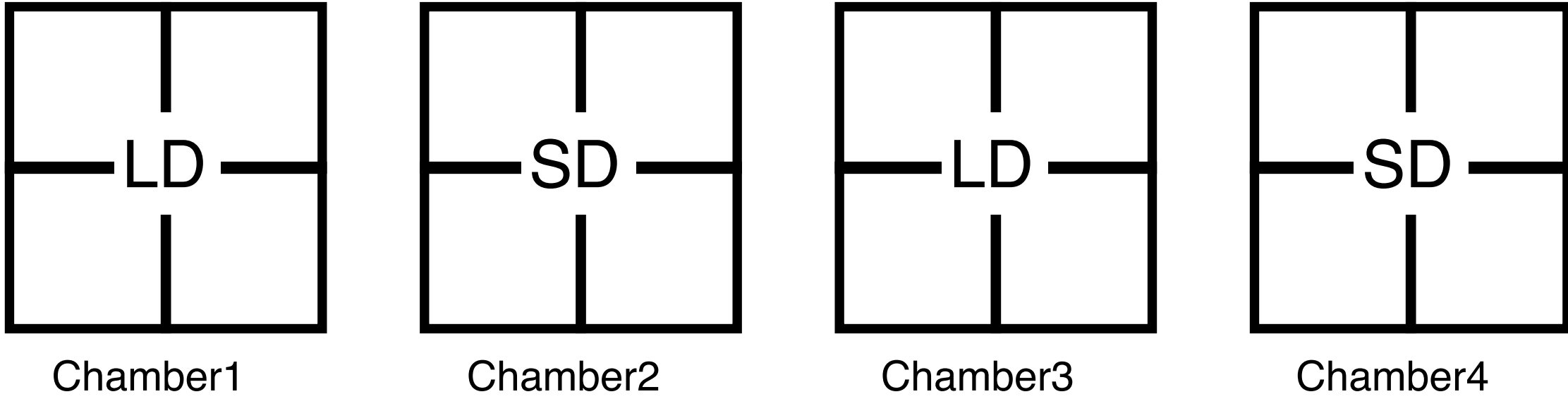
1) Genotype



Plant

Chamber

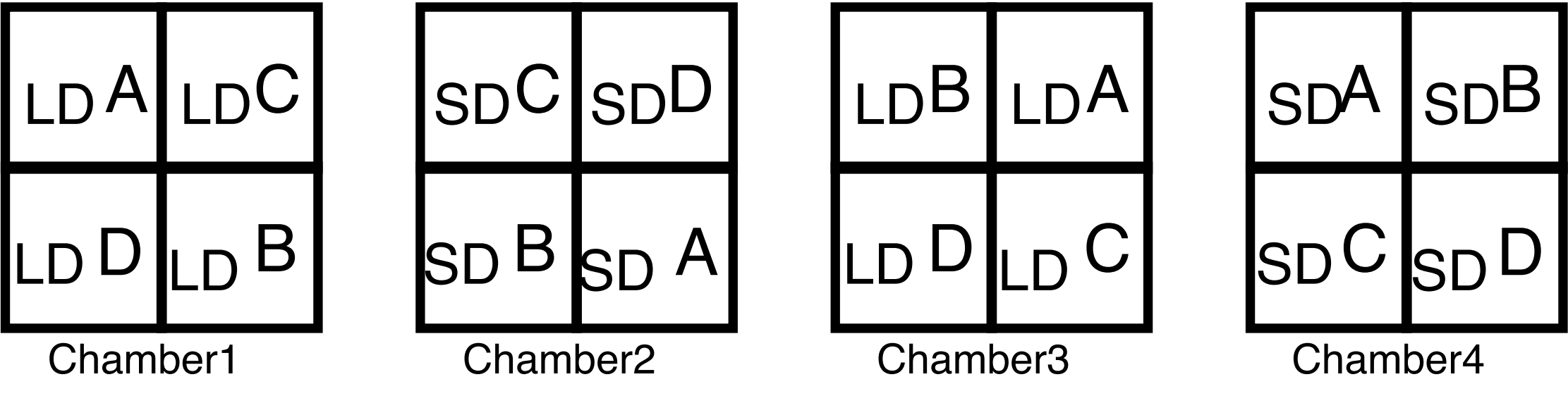
2) Daylength



Chamber

None

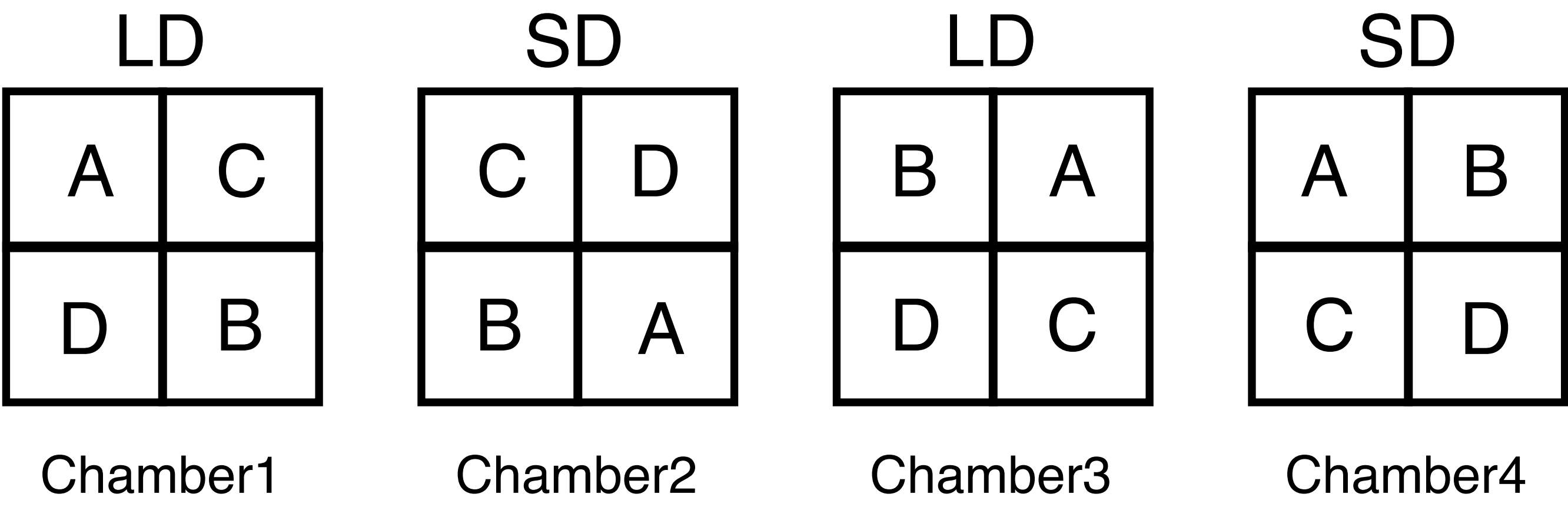
3) Genotype:Daylength



Plant

Chamber

Incomplete Block



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 | Chamber |
| Design | Chamber | 4 | | |
| | Plant | 16 | | |
| | Geno:Chamber | 16 | | |
| | Geno:Dayl:Chamber | 16 | | |
| Response | Days | 16 | | |

lmer(Days~Geno+Dayl+Geno:Dayl +(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