Split-plot designs : the Swiss-army knives for experimental designs.

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Detecting and/or setting-up split-plots

- Split-plots involve at least two factors

- **If** the combination of the levels of the two factors *are NOT randomly assigned* in the 'physical' experimental units,

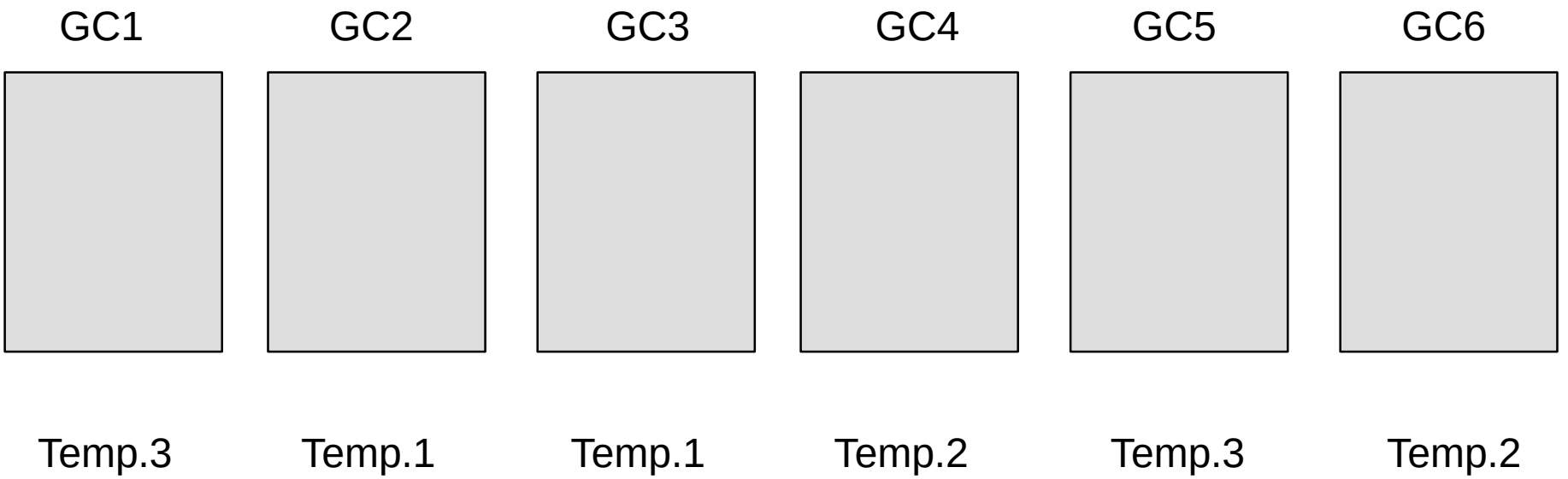
  Or

- **If** the assignment of the two factors are done *in two separate steps*,

  then this is a split-plot

Response to three temperature of four cultivars

- You use 6 different growth chambers. There are the ***whole plots***.
- Randomly assign each temperature to two of the whole plots. Temperature is the ***whole plot factor***.

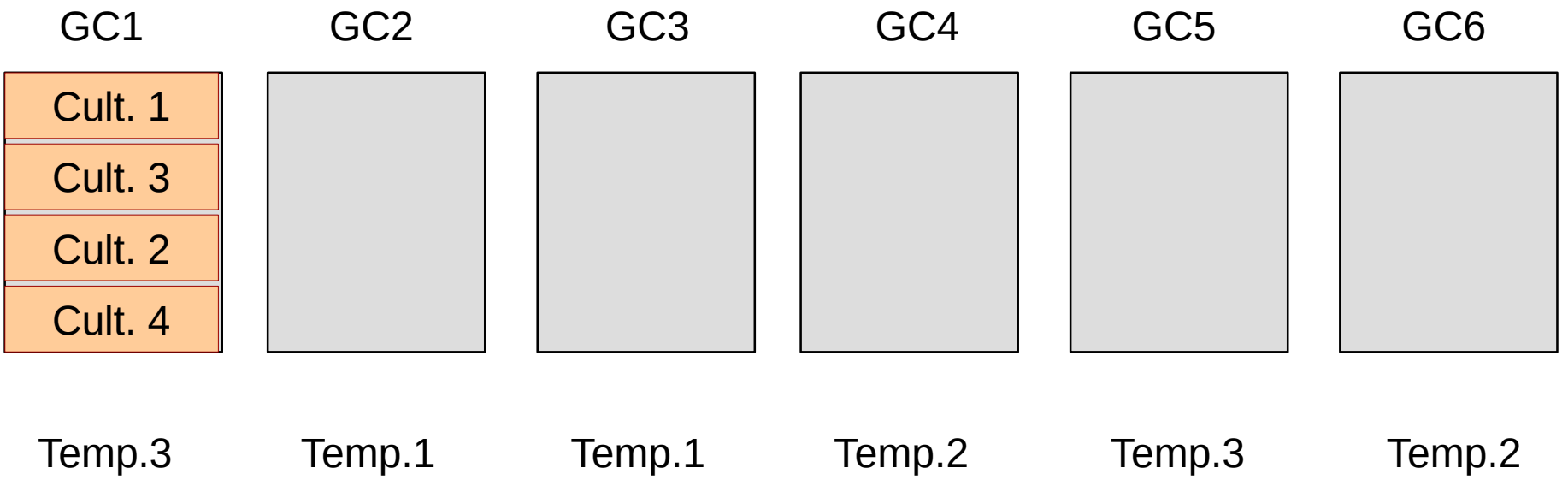


This is the FIRST of step randomisation : the whole-plot factor is randomised among the whole-plots.

 # whole plots > # whole plot factor levels

Response to three temperature of four cultivars

- Divide each whole plot (growth chamber) into four ***split plots***.
- Randomly assign the four cultivars to the four split plots, with a **separate, independent randomization** in each whole plot.
  Cultivar is the ***split-plot factor***.



Response to three temperature of four cultivars

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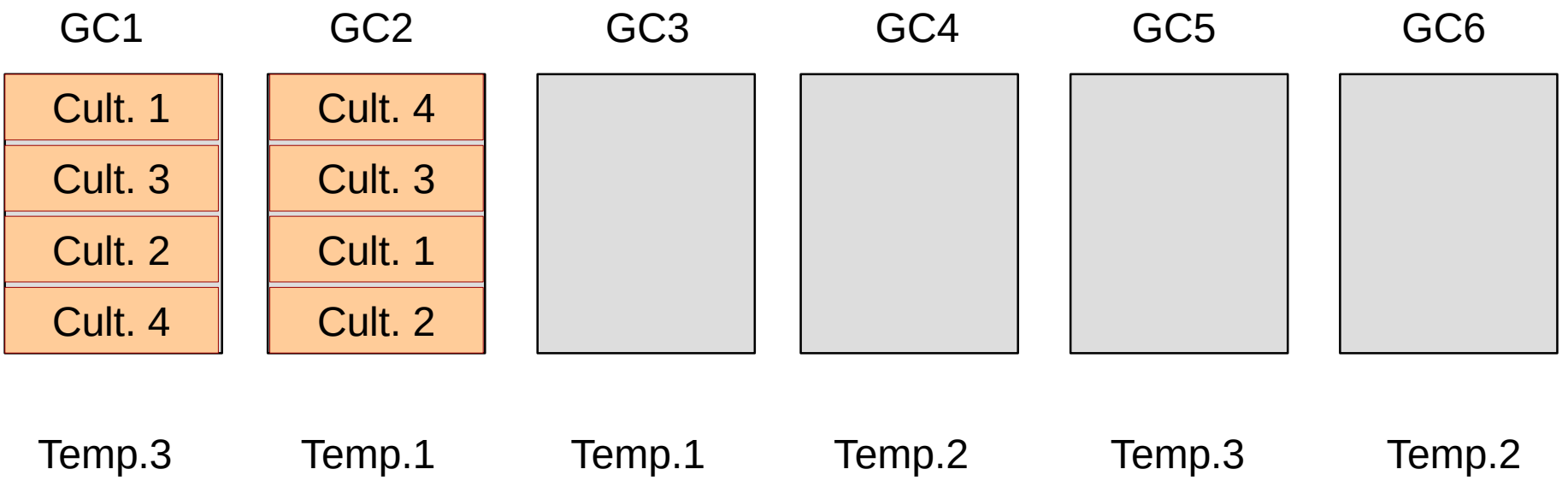

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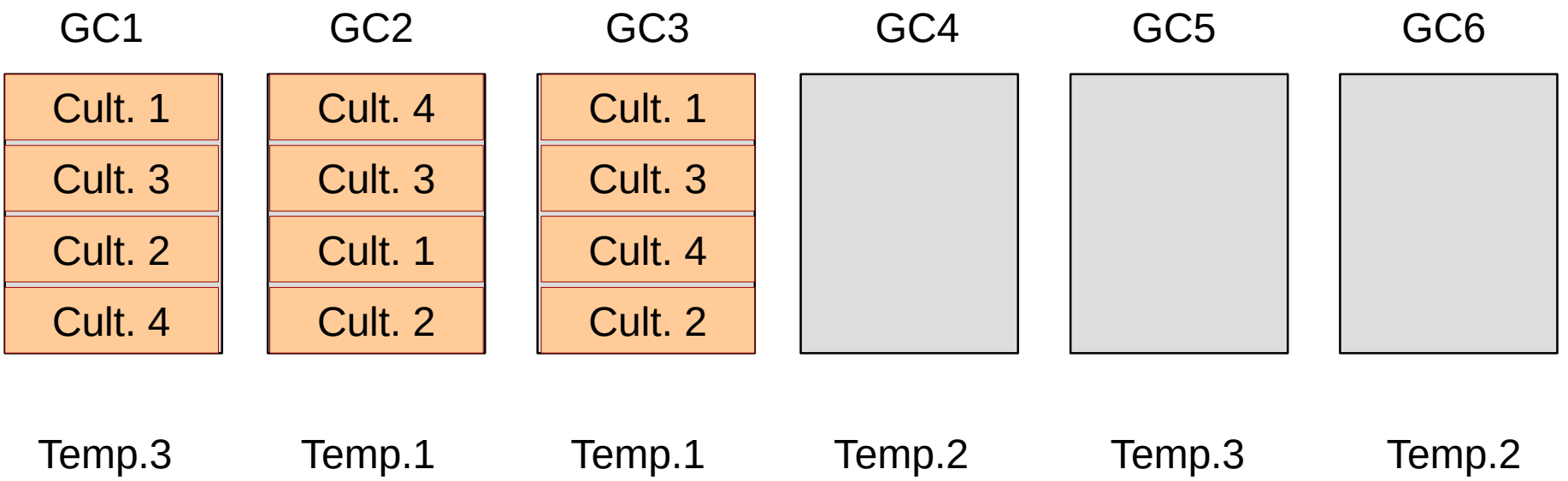
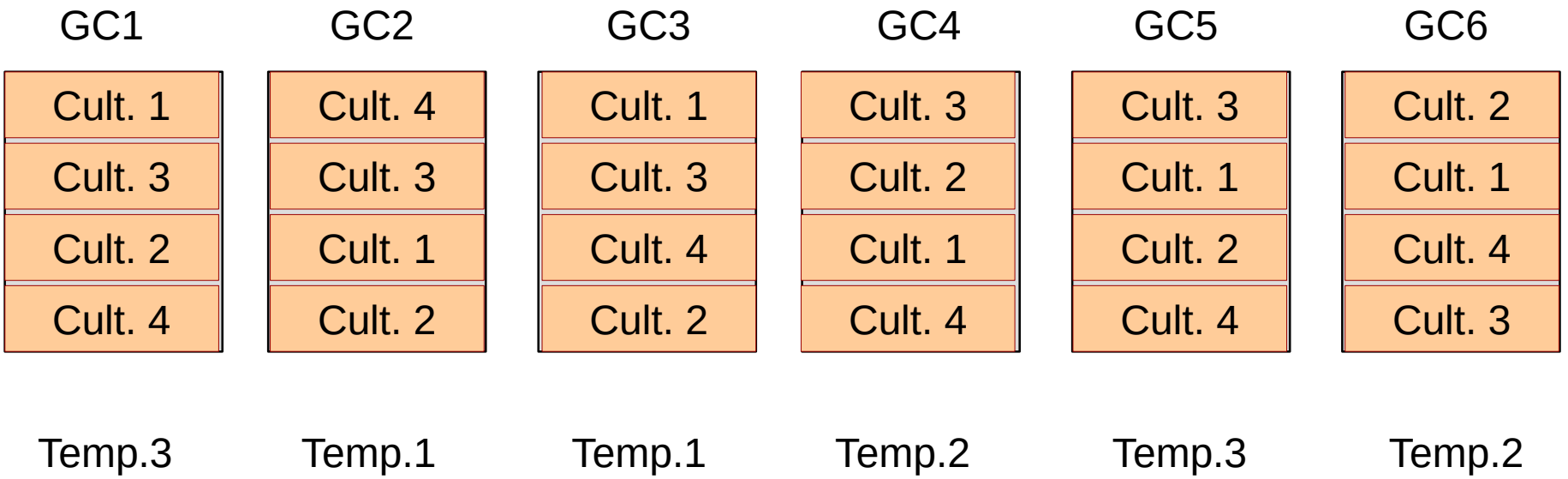


Response to three temperature of four cultivars



This is the SECOND step of randomisation : the split-plot factor is randomised among the split-plots.

 # split plots >> # split plot factor levels

Is the below design a split-plot ?

a_1	b_1
a_1	b_3
a_1	b_4
a_1	b_2

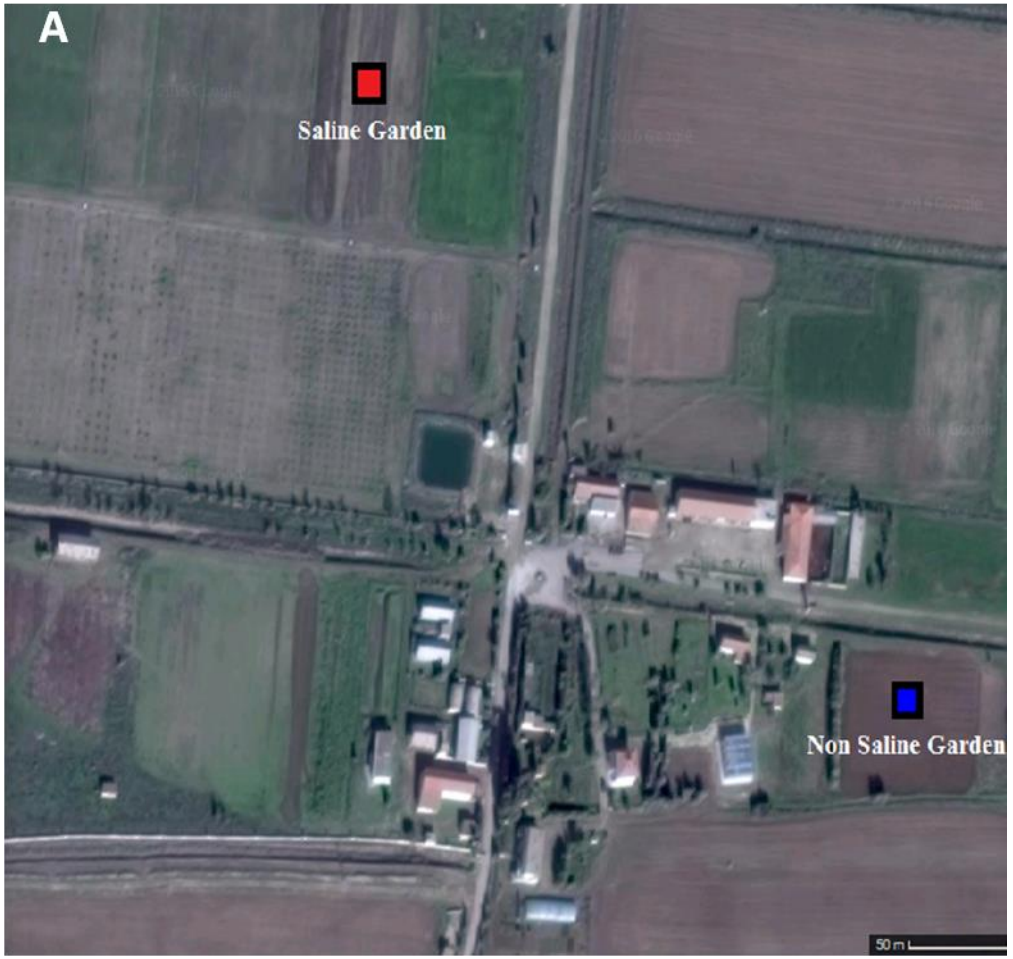
a_2	b_3
a_2	b_2
a_2	b_1
a_2	b_4

a_3	b_3
a_3	b_1
a_3	b_2
a_3	b_4

Example : factor A is watering factor ,
a1 : one growth chamber with strain1
a2 : one growth chamber with strain3
a3: one growth chamber with strain2

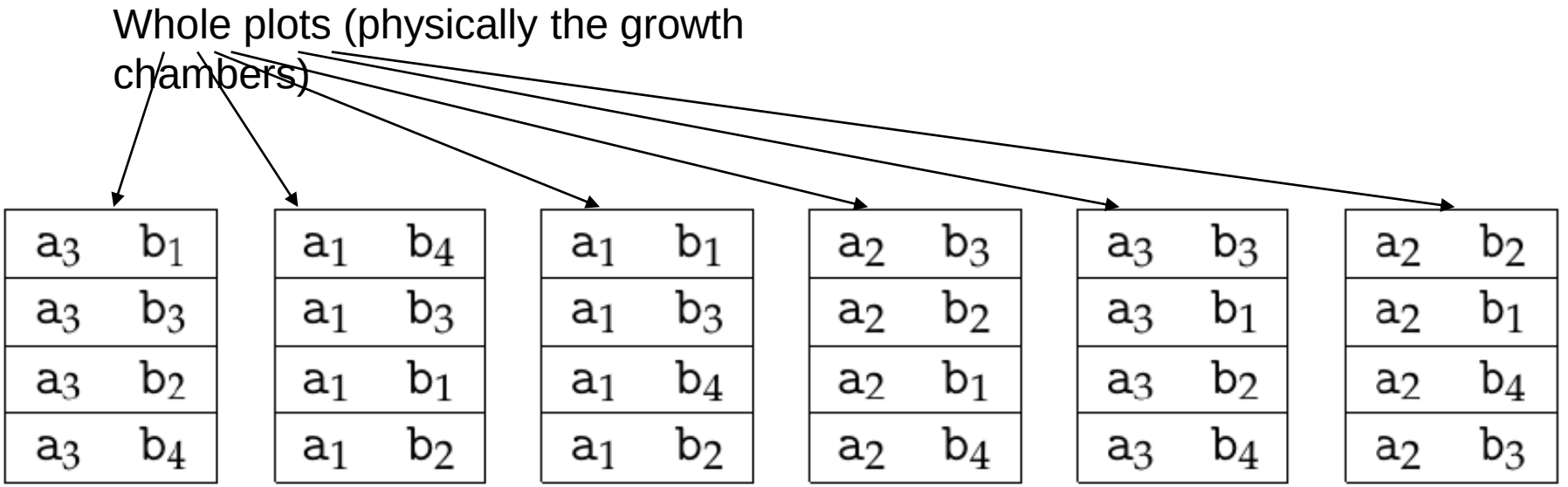
WHY ?

Is the below experimental site usefull for split-plot designs ?



WHY ?

Whole plots (physical) are different from whole-plot factor (a concept)



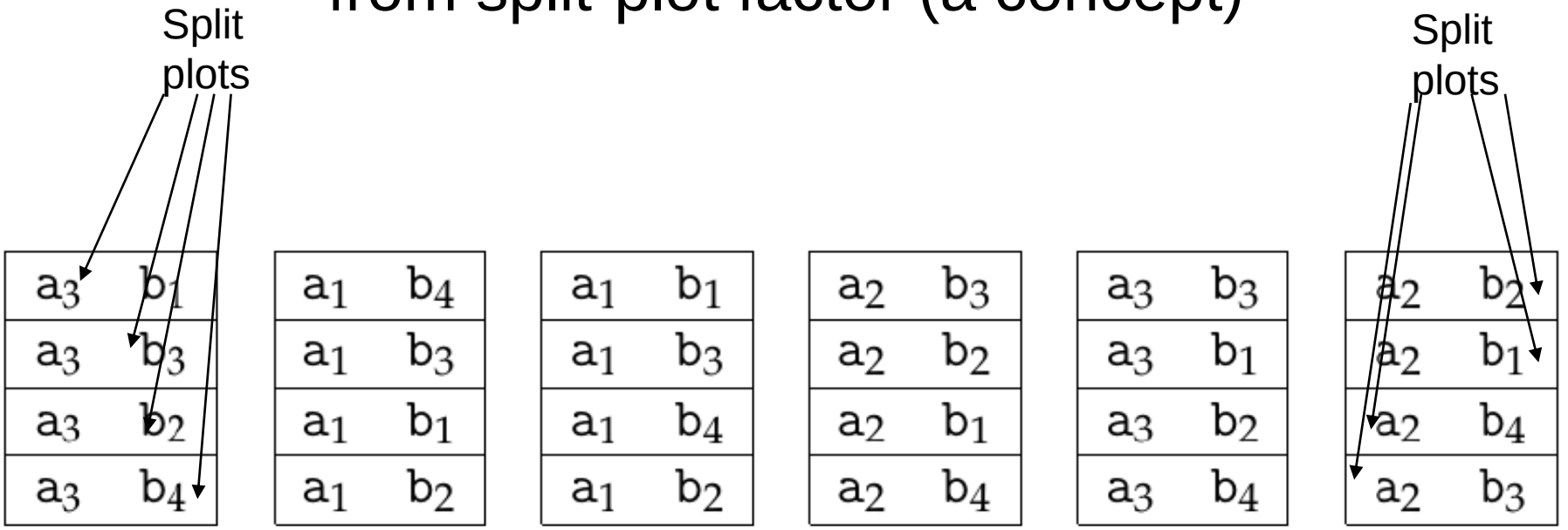
Here 6 whole plots, such that 2 evaluations of each level of whole-plot factor

Whole-plot factor : factor A with three levels

The whole-plot factor is randomised among the whole-plots

There is a clear difference between whole-plots and whole-plot factor, that will be indicated in ANOVA models

split plots (physical) are different from split-plot factor (a concept)



Here 24 sub plots, such that 6 evaluations of each level of split-plot factor

Split-plot factor : factor B with four levels

The split-plot factor is randomised among the sub plots

There is a clear difference between sub plots and split-plot factor, that will be indicated in ANOVA models

Two levels of randomization : two different errors

a_3	b_1
a_3	b_3
a_3	b_2
a_3	b_4

a_1	b_4
a_1	b_3
a_1	b_1
a_1	b_2

a_1	b_1
a_1	b_3
a_1	b_4
a_1	b_2

a_2	b_3
a_2	b_2
a_2	b_1
a_2	b_4

a_3	b_3
a_3	b_1
a_3	b_2
a_3	b_4

a_2	b_2
a_2	b_1
a_2	b_4
a_2	b_3

- Whole plot factor level a3 is assessed in whole plot 1 & whole plot 5
- Imagine there is NO experimental variability :
  - Mean of whole-plot 1 = mean of whole plot 5
    because the same cultivars b1..b4 are tested in both whole-plots.
- If there IS a difference between the mean of whole-plot 1 and the mean of whole-plot 5
  - This difference is due to experimental error due to whole-plots
    because they both contain level a3 and levels b1..b4
- ***Whole plots are the error term for whole-plot factor***

A basic split-plot has “two experiments” in one

1. at the whole-plot level (the growth chambers)

’Temperature’ is tested in a CRD design with 6 growth chambers, 2 measures of each of the 3 temperature

Source	df
Temperature	2 (= 3 -1)
Error (growth chambers)	3 (= 5 - 2)
Total	5 (= 6 -1)

2. at the split-plot level (the 4 places within each chambers)

’Genotype’ *and its interaction with* ‘temperature’ is tested in a RCBD design with 6 blocks (growth chambers)

Source	df
Blocks (Growth chambers)	5 (= see above)
Genotype	3 (= 4-1)
Genotype x Temp	6 (= 3 x 2)
Error (split-plots)	9 (= 23 – 5 – 3 - 6)
Total	23 (= 24 -1)

Preliminary analysis to provide easy way to write the model and identify errors for comparisons

Source	df
Temperature	2 (= 3 -1)
Error (per growth chambers)	3 (= 5 - 2)
Total	5 (= 6 -1)

Source	df
Blocks (Growth chambers)	5 (= see above)
Genotype	3 (= 4-1)
Genotype x Temp	6 (= 3 x 2)
Error (per split-plot)	9 (= 23 – 5 – 3 - 6)
Total	23 (= 24 -1)

$$y_{ijk} = \mu + \alpha_i + \eta_{k(i)} + \beta_j + \alpha\beta_{ij} + \epsilon_{ijk}$$

Response to three temperature of four cultivars

GC1	GC2	GC3	GC4	GC5	GC6
Cult. 1	Cult. 4	Cult. 1	Cult. 3	Cult. 3	Cult. 2
Cult. 3	Cult. 3	Cult. 3	Cult. 2	Cult. 1	Cult. 1
Cult. 2	Cult. 1	Cult. 4	Cult. 1	Cult. 2	Cult. 4
Cult. 4	Cult. 2	Cult. 2	Cult. 4	Cult. 4	Cult. 3
Temp.3	Temp.1	Temp.1	Temp.2	Temp.3	Temp.2

Split plot with one whole-plot factor, one split-plot factor, and CRD at the whole-plot level

$$y_{ijk} = \mu + \alpha_i + \eta_{k(i)} + \beta_j + \alpha\beta_{ij} + \epsilon_{ijk}$$

Exercise 1 :

A young scientist / engineer collected 6 new strains of his/her favorite pathogen/pest/symbiont

The scientist ought to test the effect of these strains on a panel of 3 reference cultivars. The value will be the mean AUDPC of 8 plants per cultivar

For practical reasons due to the infection process,
he/she needs to infect and grow all cultivars in a single tray, for a given strain,

As a good scientist / engineer, he/she decided to do four (simultaneous) replicates of the analysis

Experiments are conducted in a growth chamber with 5 shelves.
He/she can not ignore that the light and temperature are not really identical between the shelves

Set-up an experimental design, given the constraints

Compute the preliminary ANOVA table (df & error terms)

White-board to set-up the design with attendees

“canonical” split-plot with one whole-plot factor, one split-plot factor and CRD at the whole-plot factor

A basic split-plot has “two experiments” in one

1. at the whole-plot level (the trays)

’Strain’ is tested in a RCDB design with 4 measures of each of the 6 strains

Source	df
Blocks (the shelves)	3 (= 4-1)
Strain	5 (= 6 -1)
Error (the trays)	15 (= 23 – 3 – 5)
Total	23 (= 24 -1)

2. at the split-plot level (the 3 split-plots within each tray)

’Genotype’ *and its interaction with* ‘Strain’ is tested in a RCBD design with 18 blocks (trays)

Source	df
Blocks (trays)	23 (= see above)
Genotype	2 (= 3 - 1)
Genotype x Strain	10 (= 2 x 5)
Error (split-plots)	36 (= 71 – 23 – 2 - 10)
Total	71 (= 72 -1 = (4 x 6 x 3) – 1))

Split-plot designs : the Swiss-army knives for experimental designs.

Fancier split-plot designs

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The whole-plot “factor” may be actually organised following other designs

Field natural infestations and managing heterogeneity

* In order to investigate the effect of three different strains of pathogens on 20 plant varieties

* and to control in the fields for

1. field natural infestation by Orobanche spp (a gradient)

2. soil moisture gradient, that is approx. perpendicular to infestation gradient

* Infestation protocol requires to proceed by plots : no possibility to randomize (varieties x strain) plots

The differences in responses of the plant varieties and interaction with

Whiteboard to set-up the design with attendees

split-plot with one whole-plot factor, one split-plot factor and Latin-Square at the whole-plot factor

Preliminary computations with attendees

1. at the whole-plot level : a Latin Square for ‘Strain’ effect

Source	df
Rows (moisture)	2 (= 3 -1)
Columns (O. infest.)	2 (= 3 -1)
Strains	2 (= 3 -1)
Error (whole plots)	2 (= 8 – 2 - 2 -2)
Total	8 (= 9 -1)

2. at the split-plot level (the 10 split-plots within each whole-plot)
’Genotype’ *and its interaction with* ‘Strain’ is tested in a RCBD design with 9 blocks

Source	df
Blocks (Whole plots, see above)	8 (= see above)
Genotype	19 (= 20 - 1)
Genotype x Strain	38 (= 19 x 2)
Error (split-plots)	114 (= 179 – 8 – 19 - 38)
Total	179 (= 180 -1 = (20 x 3 x 3) – 1))

$$y_{crijk} = \mu + Row_r + Col_c + Strain_i + \eta_{k(cri)} + Genotype_j + Genotype_j \cdot Strain_i + \epsilon_{crijk}$$

The split-plot “factor” may be actually a combination of two factors

Salt stress, symbionts and pathogens

In order to investigate the effect of two different types of salt stress and 6 different combinations of symbionts and pathogens
on the response of plants given the salt stress, 4 trays in a greenhouse were randomized on the two types of salt stress so that 2 trays were given each type.

Each tray is divided in 6 micro-sites that are used in the combination experiment. Two types of pathogenic conditions (infected or control) were used along with 3 different symbiotic conditions (control, species 1, species 2).
The biomass of the plants was determined after 2 months of growth.

Whiteboard to set-up the design with attendees

Split plot with one whole-plot factor, two split-plot factors, and CRD at the whole-plot level

$$Y_{ijkl} = \mu + \alpha_i + \eta_{l(i)} + \beta_j + \gamma_k + \beta\gamma_{jk} + \alpha\beta_{ij} + \alpha\gamma_{ik} + \alpha\beta\gamma_{ijk} + \epsilon_{l(ijk)}$$

Preliminary computations

1. at the whole-plot level (the trays)

'Salt' is tested in a CRD design with 2 trays thus 2 measures of each of the 2 salt levels

Source	df
Salt	1 (= 2 -1)
Error (trays)	2 (= 3 - 1)
Total	3 (= 4 -1)

2. at the split-plot level (the 6 micro-sites places within each tray)

'Symbiosis', 'Inoculation', their interaction and the interactions with 'Salt' are tested in a RCBD design with 4 blocks (trays)

Source	df
Blocks (trays)	3 (= see above)
Symbiosis	2 (= 3-1)
Inoculation	1 (= 2 -1)
Symb. X Inoc.	2 (= 2 x 1)
Symb x Salt	2 (= 2 x 1)
Inoc x Salt	1 (= 1 x 1)
Symb x Inoc x Salt	2 (= 2 x 1 x 1)
Error (split-plots)	10 (= **23** – 3 – 2 – 1 - 2 – 2 – 1 – 2)
Total	**23** (= 24 -1 = (4x6) - 1)

Design with Split-split-plot

NIBIO

Nitrogen availability from peas and fava bean as pre-crops to broccoli followed by lettuce, in Norwegian organic field trial

Randi Seljåsen[1], Torfinn Torp[2], Ingunn M. Vågen[1]
NIBIO, Norwegian Institute of Bioeconomy Research, Division of food production and society

Poster nr 135

THE OBJECTIVES

- Investigate the available nitrogen from legume pre crops to following crops
- Nitrogen dynamics and yield effects was studied for main crop of broccoli followed by lettuce.

METHODS

The experiment was arranged over **2 years as a stepwise 'split-split plot design' with 4 blocks and 3 factors:**

F1: Pre crop
- 2 levels **Pea** or **Fava bean** (spring 2014, 89.6 m^2)

F2: 'Rest-covercrop' autumn treatment
- 4 combinations of **legume residue incorp.** and winter **cover crop,** autumn 2014, 22.4m^2

F3: Manure
- **+ / - Chicken manure** (80 kg N ha^{-1}) spring 2015, 10.2 m^2, before broccoli crop

Poster 135: Nitrogen availability from pea and fava bean to following crops of broccoli and lettuce, R. Seljåsen et al, NIBIO Norway.

Split-split-plot with RCBD at the whole-plot level

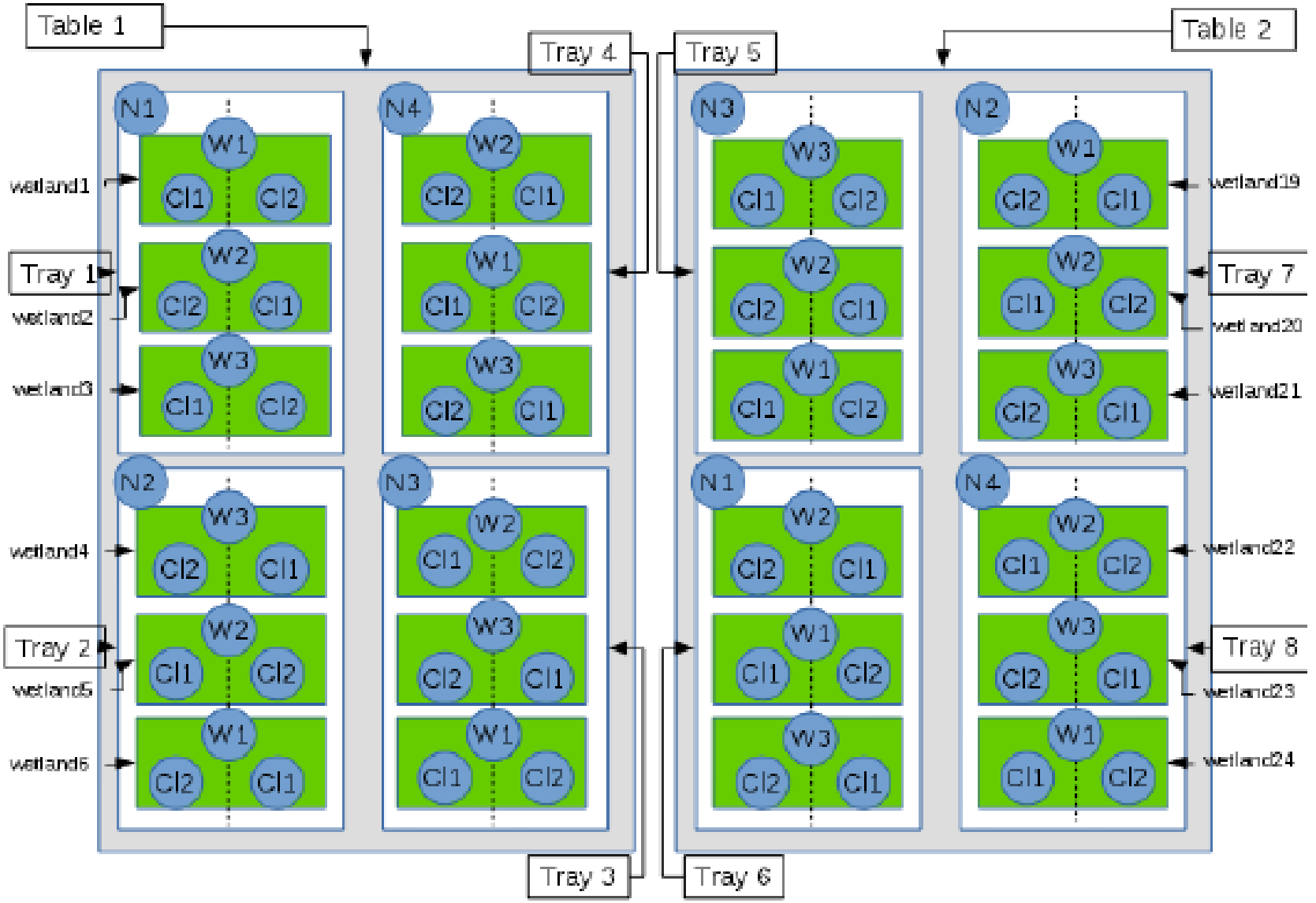
Context: *An experiment studies the effect of nitrogen and weeds on plant growth in wetlands. Effect of four levels of nitrogen, three weed treatments (no additional weeds, addition of weed species 1, addition of weed species 2), and two herbivory treatments (clipping and no clipping) were investigated.*

Experimental design includes eight trays; each tray holds three artificial wetlands consisting of rectangular wire baskets containing wetland soil. The trays are full of water, so the artificial wetlands stay wet. All of the artificial wetlands receive a standard set of seeds to start growth.

Four of the trays are placed on a table near the door of the greenhouse, and the other four trays are placed on a table in the center of the greenhouse.
On each table, we randomly assign one of the trays to each of the four nitrogen treatments.
Within each tray, we randomly assign the wetlands to the three weed treatments.
Each wetland is split in half. One half is chosen at random and will be clipped after 4 weeks, with the clippings removed; the other half is not clipped.

After 8 weeks, we measure the fraction of biomass in each wetland that is non-weed as our response.

Split-split-plot with RCBD at the whole-plot level



Split-split-plot with RCBD at the whole-plot level

- Let us follow the path of randomization:
  - Position on tables in the greenhouse is a block factor (center / door)
  - Trays are whole plots, and “nitrogen level” is the whole-plot factor.
  - Wetlands are split plots and “weed treatment” is the split-plot factor.
  - Wetland-halves are split-split plots and “clipping” is the split-split-plot factor.

$$
\begin{aligned}
y_{ijk} = \mu + b_z + \alpha_i + \eta_{l(i)} \\
+ \beta_j + \alpha\beta_{ij} + \zeta_{l(ij)} \\
+ \gamma_k + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + \epsilon_{l(ijk)}
\end{aligned}
$$

At keyboards!

Preliminary computations

1. at the whole-plot level (the trays)

'Nitrogen' is tested in a RCBD design with 2 tables thus 2 measures of each of the 4 N levels

Source	df
Blocks (Table)	1 (= 2 - 1)
Nitrogen	3 (= 4 -1)
Error (trays)	3 (= 7 – 3 - 1)
Total	7 (= 8 -1)

2. at the split-plot level (the wetlands within each tray)

'Weed treatment' and its interactions with 'Nitrogen' are tested in a RCBD design with 8 blocks (the trays)

Source	df
Blocks (trays)	7 (= see above)
Weed trt	2 (= 3 - 1)
Weed X Nitro.	6 (= 2 x 3)
Error (wetlands)	8 (= 23 – 7 – 2 - 6)
Total	23 (= 24 -1 = (3x8) - 1)

Preliminary computations

2. at the split-split-plot level (the half-wetlands within each wetland within each tray)
'Clipping' and its interactions with 'Nitrogen' and "Weed treatment" and above interaction are tested in a RCBD design with 24 blocks (the wetlands)

Source	df
Blocks (wetlands)	23 (= see above)
Clipping trt	1 (= 2 - 1)
Clipping X Weed	2 (= 1 x 2)
Clipping X Nitro.	3 (= 1 x 3)
Clipping X Weed X Nitro.	6 (= 1 x 2 x 3)
Error (half-wetlands)	12 (= 47 – 23 – 1 – 2 – 3 – 6)
Total	47 (= 48 -1 = (2 x 3 x 8) - 1)