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Design Tableau: An Aid to Specifying the Linear Mixed Model for a Comparative Experiment

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Design Tableau: An aid to specifying the linear mixed model for a comparative experiment

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1 Introduction

The design and analysis of comparative experiments has changed dramatically in recent times. There has been a move away from "text-book" designs towards complex, non-orthogonal designs. At the same time, analysis of variance (ANOVA) techniques have been superseded by the use of linear mixed models (LMM). The latter have the advantage of accommodating non-orthogonality and of allowing more complex variance models, which may be beneficial for improving the efficiency of treatment comparisons or for providing a more plausible structure. However, this flexibility has come at a cost, since in the transition from ANOVA to LMM, some of the key principles in the analysis of comparative experiments are often neglected. In order to address this we have developed a simple but general approach for specifying the LMM for a comparative experiment. In doing so, we defer to the seminal work of John Nelder and Rosemary Bailey and extend it to encompass multi-environment trials and multi-phase experiments. We call the approach "Design Tableau", since information on the experimental design and measurement process that is pivotal to specification of the LMM is conveyed in tabular form.

This document provides an outline of the Design Tableau (DT) approach as developed thus far. In Section 2 we list some definitions and concepts from Rosemary Bailey's book ("Design of Comparative Experiments", 2008) that are pivotal to DT. These are extended and modified as neccesary for DT in Section 3. Section 4 lists the steps in the DT process. Finally, as an Appendix, we include the inaugural presentation of the Design Tableau approach which was as a key-note address at the CEN-ISBS conference in Vienna, 2017.

2 Key definitions and concepts from Rosemary Bailey (RB)

The definitions and concepts for Design Tableau borrow very heavily from Rosemary Bailey's "Design of Comparative Experiments" (2008). The following provides a list of the relevant concepts from the book with links to the page numbers.

1. An experimental unit is the smallest unit to which a treatment can be applied (p8)

2. A treatment is the entire description of what can be applied to an experimental unit (p8)

3. An observational unit is the smallest unit on which a response will be measured. (p8) It is often called a *plot*. (p10)

4. Plot factors: In the context of the rye-grass example, "Thus field and strip are relevant factors on Ω even before we apply the treatments, so we call them *plot factors*." (p170)

5. Treatment factors: In the context of the rye-grass example, "The treatments consist of all combinations of the factors cultivar and nitrogen. ... Thus cultivar and nitrogen are *treatment factors*, as is T itself." (where T is the wedge of cultivar and nitrogen). (p170).

6. Treatment structure: meaningful ways of dividing up the set of all treatments. (p12). RB uses words to describe structures eg. "unstructured", "all combinations of two factors".

7. Plot structure: meaningful ways of dividing up the set of all plots, ignoring treatments. (p12) RB uses words to describe structures eg. "unstructured", "blocks".

8. Design function: manner in which treatments are allocated to plots (p13). "Although we speak of allocating treatments to plots ... [it is actually the case that] the plot ω is allocated treatment $T(\omega)$ ... because each plot can receive only one treatment.". Important to remember that a treatment is the combination of levels of all treatment factors; and a plot is an observational (not experimental) unit.

9. "The method of randomization is usually dictated by the plot structure, but occasionally we use the method of randomization to define the plot structure." (p15).

10. Response: "The response on plot ω is a random variable Y_ω whose observed value after the experiment is y_ω. Thus we have a data vector $\boldsymbol{y}$ which is a realization of the random vector $\boldsymbol{Y}$." (p14). Note that RB uses the term "response" for both the random variable (as she does here)

and also the measurement (eg. in caption of Table 13.5: "Treatments and responses in Example 13.6".)

11. Aliasing: "Let F and G be factors on the same set (ie both treatment factors or both plot factors). Then F and G are aliased if F and G are the same apart from the names of their levels." (p170). Why do they need to be on the same set? Because RB then says "If a treatment factor is aliased with a plot factor, it may indicate false replication." (p170) In which case "... there is no residual mean square for testing significance or for examining variance" (p200)

12. Universal factor, U: has a single class (level) ie. all plots have the same level (p171)

13. Equality factor, E: has a class (level) for every plot (p171)

14. "... the plot structure makes no difference to the expectation model but does determine the covariance matrix. Thus we hope for a plot structure which defines a covariance matrix whose strata can be determined." (p193)

15. Definition for a set of treatment factors to be orthogonal - see p190

16. Definition for a set of plot factors to be orthogonal - see p194. This requires the 3 conditions for an orthogonal treatment structure plus (a) "E must be included, so that we obtain a decomposition of the whole space.", (b) "all factors must be uniform [(equal size) ... otherwise] we cannot relabel them by randomization." and (c) "if F and G are plot factors then so must F^G."

17. "If plots are structured [in any way] ... the universal factor must be considered both a treatment factor and a plot factor." (p189)

18. Definition for a design to be orthogonal - see p198. Requires both the treatment and plots structures themselves to be orthogonal plus a third criterion.

3 Key definitions and concepts for Design Tableau (DT)

The definitions and concepts for DT borrow very heavily from RB. A crucial difference, however, is that RB is concerned with the design of comparative experiments whereas we are concerned with the analysis of a comparative experiment, *given* the design. I believe this requires some slightly different (stronger) definitions, particularly for plot factors.

1. An experimental unit is the smallest unit to which a treatment can be applied. Same as RB 1.

2. A treatment is the entire description of what can be applied to an experimental unit. Same as RB 2.

3. An observational unit is the smallest unit on which a response will be measured and we will call it a *plot*. Same as RB 3.

4. Design function: manner in which treatments are allocated to plots. Same as RB 8. Important to note this is allocation to observational units not experimental units.

5. Measurement process: manner in which measurements taken. This may involve sub-sampling which may not be accounted for in design function.

6. Plot factors: factors that define meaningful ways of dividing up the set of all plots *and are utilised in the design function or measurement process*. RB definition of plot factors is only in the context of an example but may be derived from RB 7. I have done this here, but with a key additional condition that these factors must also be used as part of the allocation of treatments or the measurement process. This is commensurate with the second scenario in RB 9 which is relevant for our aim of determining a LMM given an experimental design.

7. Treatment factors: factors whose combinations form the set of treatments. Essentially same as RB 5.

8. Treatment structure: meaningful ways (contrasts of interest?) of dividing up the set of all treatments. Same as RB 6 but we will use a model formula not words to describe it.

9. Plot structure: the division of the set of all plots commensurate with the design function and measurement process. This is a stricter version of RB 7 ie. not just a "meaningful" division (also see DT 6). Once again we use a model formula not words to describe it.

10. Variable: a variable has two defining characteristics, namely (a) it is an attribute that describes a person, place, thing, or idea and (b) the value of the variable can "vary" from one entity to another (StatTrek.com)

11. Response variable: variable under study ie. whose response to the treatments is of interest

12. Anatomical: something that relates to living things and how they are structured (yourdictionary.com)

13. Anatomical variables: variables that relate to the scientific question but are not treatment factors (so were not applied/randomised). The effects of these variables, and potential interactions with treatment factors, are of interest to the researcher. They will therefore be added to the LMM as part of the treatment structure. Note that plot factors cannot be anatomical variables but it is posible for a plot factor to be aliassed with an

anatomical variable. Multi-environment trials provide a classic example, with "Experiment" being a plot factor and "Environment" an anatomical variable (factor) with which it is aliassed.

14. Extraneous variables: variables that may influence the response, but are not of interest. These variables can be added to the LMM in order to reduce error.

15. We will define *two* universal factors, ie. one for plots ("U") and one for treatments ("1"). They have the same values so are aliased (RB 11). This will allow generalistaions for aliasing between plot and treatment factors.

4 Steps for Design Tableau

1. Define treatments (DT 2) and list treatment factors (DT 7)

2. Define plots (observational units, DT 3) and list plot factors (DT 6). It must be possible to index observational units using combinations of levels of plot factors (also see step 7 below)

3. Describe design function (DT 4) and measurement process (DT 5) and thence experimental unit (DT 1). May be easiest to work upwards from observational unit (E factor) towards universal factor.

4. List anatomical variables, if any (DT 13)

5. List extraneous variables, if any (DT 14)

6. Use treatment factors and anatomical variables to construct model formula for treatment structure (DT 8). Use Wilkinson and Rogers (1973) notation. Include the universal treatment factor, 1. (see RB 17). Effects represented in the treatment model formula will be included in the LMM as fixed effects.

7. Use plot factors to construct model formula for plot structure (DT 9). Combinations of levels of factors in model formula must completely index observational units. (see RB 16) Include the universal plot factor, U. (see RB 17). Effects represented in the plot model formula will be included in the LMM as random effects (see RB 14).

8. Identify obvious aliasing of factors in treatment and plot model formulae. At a minimum this will be the universal plot and treatment factors, ie. U and 1. We will fit the fixed effect "1" which means we cannot also fit U which in turn means there is no inference for testing this fixed effect. We indicate all of this by re-labeling "1" as "1[U]". In general for a treatment factor F which is aliassed with a plot factor G, we first will need to decide whether the associated effects will be fitted as fixed or random. If fixed, then we re-label F as "F[G]" (to show there can be no inference on F). (see

RB 11) If random, we re-label G as "G[F]" (to show that if the variance component for G is large it may be attributable to either G or F). If there is any aliassing, then re-write the treatment and plot model formulae using the new factor labels.

9. Construct a table (Design Tableau) listing all terms in the treatment model formula followed by terms in the plot model formulae. Indicate whether terms will be fitted in the LMM as fixed (treatment structure) or random (plot structure).

10. Fit LMM commensurate with the Design Tableau

11. Possibly modify the Design Tableau to accommodate extraneous variables; more complex variance structures etc. Note that for a classical randomisation-based analysis of an orthogonal design there will only be the single DT.

Design Tableau: An aid to specifying the linear mixed model for a comparative experiment

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Vienna

Design Tableau

Overview of talk

- Motivation
- Calf-feeding example
  - Randomisation distribution
  - Analysis of Variance (ANOVA)
  - Linear Mixed Model (LMM)
- Design Tableau approach
  - Definitions
  - Steps
  - Application to calf-feeding example
- Multi-phase experiment example
- Design Tableau approach for multi-phase experiments
- Summary
  - Orthogonal designs
  - Non-orthogonal designs
  - Model based analysis and design

Motivation

Design and analysis of comparative experiments

- Design and analysis of comparative experiments has changed dramatically in recent times
- A move away from “text-book” designs towards complex, non-orthogonal designs
- With proliferation of linear mixed models (LMM) software (ASReml-R, SAS, lme), a move away from analysis of variance (ANOVA) techniques
- LMM have advantage of accommodating non-orthogonality
- LMM have advantage of allowing more complex variance models. Beneficial either for
  - improving efficiency of treatment comparisons or
  - providing a more plausible structure

Motivation

Design and analysis of comparative experiments

- But, this flexibility has come at a cost
- In transition from ANOVA to LMM, we seem to have lost sight of some of the key principles for the analysis of comparative experiments
- Literature full of examples of the mis-use of LMM for comparative experiments. Some common flaws include
  - failing to recognise pseudo (or false) replication
  - testing/dropping model terms that define strata
  - providing standard errors for means (not contrasts)
  - failing to recognise the need for negative estimates of variance components
  - failing to provide sufficient detail for reader to uncover some of these flaws!

Motivation

Design and analysis of comparative experiments

- Our motivation behind Design Tableau was to help address these issues and re-focus on the key principles
- Design Tableau is a simple but general approach for specifying the LMM for a comparative experiment
- Relies heavily on the seminal work of John Nelder and Rosemary Bailey for the analysis of experiments with orthogonal designs
- But extends to cover
  - complex experiments with non-orthogonal designs (eg. multi-environment trials, longitudinal data)
  - complex variance modelling (model based analysis)

Text-book example (Bailey, 2008)

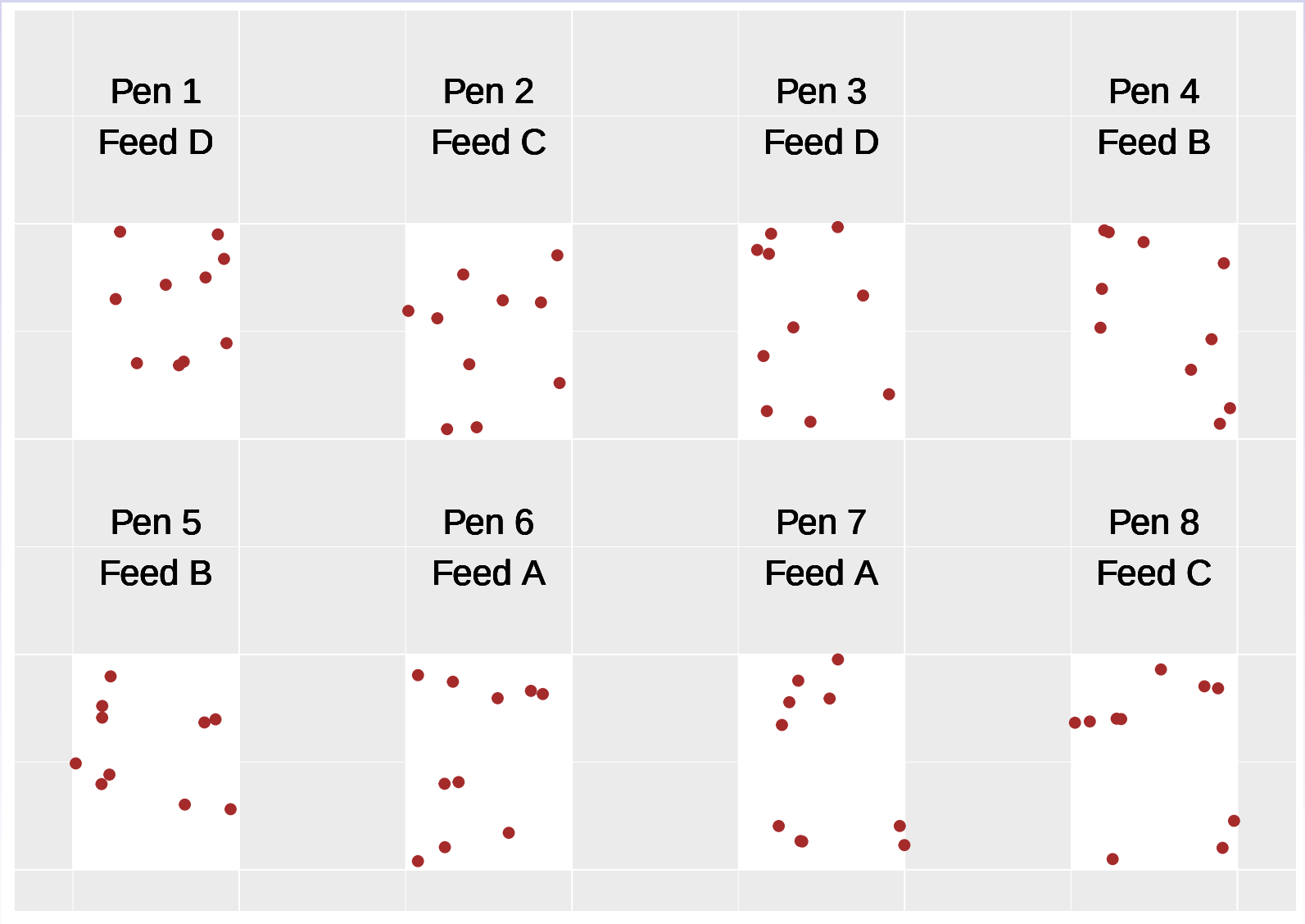
Calf feeding experiment

- Four ($t = 4$) feed treatments (A,B,C,D) are to be compared using $n = 80$ calves
- The calves are housed in $m = 8$ pens with $k = 10$ calves per pen so that $n = mk$
- Each pen allocated one of the four feeds (all calves within the pen consume the same feed)
- Calves are weighed individually at birth then at several times thereafter
- For illustrative purposes we assume variable to be analysed is average daily weight gain for each calf:

$$\boldsymbol{y} = \frac{\text{final calf weight} - \text{initial calf weight}}{\text{number of days}}$$

Text-book example (Bailey, 2008)

Calf feeding experiment



Classical analysis
Randomisation theory

- Classical analysis for comparative experiments is based on randomisation theory (Nelder, 1954)
- Data are re-randomised to the observational units and inferences are based on the observed outcome of the resultant randomisation distribution
- This provides a platform for inference that is distribution-free

Calf feeding experiment

Randomisation distribution: the null experiment

- Consider the *null experiment*: all calves are assumed to receive the same treatment (Nelder, 1954, 1965a)
- To obtain the moments of the randomisation distribution the observed data are considered as given or known
- Let $x_{ij},\ i = 1 \ldots m, j = 1 \ldots k$ be the observed datum from the j^{th} calf in the i^{th} pen
- From these numbers form a set of random variables y_{ij} by
  - Choose a pen at random; re-order members at random to give $y_{11}, \ldots, y_{1k}$
  - Repeat procedure with another pen to give $y_{21}, \ldots, y_{2k}$
  - Repeat for all other pens

Calf feeding experiment

Randomisation distribution: the null experiment

- The (null) distribution of the y_{ij} is such that

$$\begin{aligned}
\mathrm{E}\left(y_{ij}\right) &= \mu_0 \\
\mathrm{var}\left(y_{ij}\right) &= \sigma_y^2 \\
\mathrm{cov}\left(y_{ij},\ y_{ib}\right) &= \rho_1\sigma_y^2 \quad (j \neq b, \text{so 2 calves in same pen}) \\
\mathrm{cov}\left(y_{ij},\ y_{ab}\right) &= \rho_2\sigma_y^2 \quad (i \neq a, \text{so 2 calves in different pens})
\end{aligned}$$

- In vector notation, and assuming that the data are ordered as calves within pens

$$\begin{aligned}
\mathrm{E}\left(\boldsymbol{y}\right) &= \mu_0\mathbf{1}_n \\
\mathrm{var}\left(\boldsymbol{y}\right) &= \sigma_y^2\left[(1-\rho_1)\boldsymbol{I}_m \otimes \boldsymbol{I}_k + \rho_2\boldsymbol{J}_m \otimes \boldsymbol{J}_k + (\rho_1-\rho_2)\boldsymbol{I}_m \otimes \boldsymbol{J}_k\right]
\end{aligned}$$

where $\boldsymbol{J}_m$ is an $m \times m$ matrix with all elements equal to 1

Calf feeding experiment

Randomisation distribution and ANOVA

- Null Analysis of Variance (ANOVA) is built up by forming *strata* which are defined as the eigenspaces of $\mathrm{var}\,(\boldsymbol{y})$
- For calf experiment there are 3 eigenspaces, with dimensions $1,\ (m-1)$ and $m(k-1)$ and eigenvalues
  - $\xi_0 = \sigma_y^2(1-\rho_1) + \sigma_y^2 k(\rho_1 - \rho_2) + \sigma_y^2 mk\rho_2$
  - $\xi_1 = \sigma_y^2(1-\rho_1) + \sigma_y^2 k(\rho_1 - \rho_2)$
  - $\xi_2 = \sigma_y^2(1-\rho_1)$
- These will be called the “mean”, “pens” and “calves” strata

Calf feeding experiment

Randomisation distribution and ANOVA

- We can then re-express $\mathrm{var}\,(\boldsymbol{y})$ as

$$\mathrm{var}\,(\boldsymbol{y}) = \xi_0 \boldsymbol{P}_0 + \xi_1 \boldsymbol{P}_1 + \xi_2 \boldsymbol{P}_2$$

- The $\boldsymbol{P}_s,\ s = 0, 1, 2$ are orthogonal projection matrices that can be written as $\boldsymbol{K}_s \boldsymbol{K}_s^\top$

Stratum	$\boldsymbol{P}_s$	$\boldsymbol{K}_s$
mean	$\boldsymbol{J}_m \otimes \boldsymbol{J}_k/(mk)$	$\mathbf{1}_m/\sqrt{m} \otimes \mathbf{1}_k/\sqrt{k}$
pens	$\boldsymbol{I}_m \otimes \boldsymbol{J}_k/k - \boldsymbol{J}_m \otimes \boldsymbol{J}_k/(mk)$	$(\boldsymbol{I}_m - \boldsymbol{J}_m/m) \otimes \mathbf{1}_k/\sqrt{k}$
calves	$\boldsymbol{I}_m \otimes \boldsymbol{I}_k - \boldsymbol{I}_m \otimes \boldsymbol{J}_k/k$	$\boldsymbol{I}_m \otimes (\boldsymbol{I}_k - \boldsymbol{J}_k/k)$

- The strata define 3 independent linear models that are obtained by applying a one-to-one transformation of the data from $\boldsymbol{y}$ to $\boldsymbol{K}^\top \boldsymbol{y}$ where $\boldsymbol{K} = [\boldsymbol{K}_0\ \boldsymbol{K}_1\ \boldsymbol{K}_2]$

Calf feeding experiment

Randomisation distribution plus treatments

- We now consider the imposition of the treatments so that $\mathrm{E}(y_{ij}) = \mu_A,\ \mu_B,\ \mu_C$ or μ_D
- Thus the first and second moments of the distribution are given by

$$\begin{aligned} \mathrm{E}(\boldsymbol{y}) &= \boldsymbol{\mu} \otimes \mathbf{1}_k \\ \mathrm{var}(\boldsymbol{y}) &= \xi_0 \boldsymbol{P}_0 + \xi_1 \boldsymbol{P}_1 + \xi_2 \boldsymbol{P}_2 \end{aligned}$$

where $\boldsymbol{\mu} = (\mu_D, \mu_C, \mu_D, \mu_B, \mu_B, \mu_A, \mu_A, \mu_C)^\top$

Calf feeding experiment

Linear models for strata

- The 3 linear models associated with the strata are defined for $\boldsymbol{K}_s^\top \boldsymbol{y}, \quad s = 0, 1, 2$ with

Stratum	$\mathrm{E}\left(\boldsymbol{K}_s^\top \boldsymbol{y}\right)$	$\mathrm{var}\left(\boldsymbol{K}_s^\top \boldsymbol{y}\right)$
mean	$\bar{\mu}\sqrt{mk}$	ξ_0
pens	$\left(\boldsymbol{\mu} - \bar{\mu}\mathbf{1}_m\right)\sqrt{k}$	$\xi_1 \boldsymbol{I}_{(m-1)}$
calves	$\mathbf{0}$	$\xi_2 \boldsymbol{I}_{m(k-1)}$

  where $\bar{\mu} = \sum_{ij} \mathrm{E}\left(y_{ij}\right)/n$

- ξ_s called stratum variances
- Typically the 3 models are represented using an ANOVA table ...

Calf feeding experiment: ANOVA table

Stratum	Source	df	ms	E(ms)	VR
mean		1			
	Mean	1	ms_M	$f_0(\bar{\mu}) + \xi_0$	
	residual	0			
pens		7			
	Feed	3	ms_F	$f_1(\boldsymbol{\mu} - \bar{\mu}\mathbf{1}_m) + \xi_1$	ms_F/ms_P
	residual	4	ms_P	ξ_1	
calves		72			
	residual	72	ms_R	ξ_2	
	Total	80			

- Using Nelder (1965b) can show that information on
  - Mean entirely in mean stratum. Obtain best linear unbiased estimate (BLUE) of mean within this stratum.
  - Feed treatment contrasts entirely in pens stratum. Obtain BLUEs of contrasts within this stratum.
- Residual mean squares provide unbiased estimates of stratum variances; cannot estimate ξ_0 so arbitrarily set $\xi_0 = \xi_1$

Calf feeding experiment: ANOVA table

Stratum	Source	df	ms	E(ms)	VR
mean		1			
	Mean	1	ms_M	$f_0(\bar{\mu}) + \xi_0$	
	residual	0			
pens		7			
	Feed	3	ms_F	$f_1(\boldsymbol{\mu} - \bar{\mu}\mathbf{1}_m) + \xi_1$	ms_F/ms_P
	residual	4	ms_P	ξ_1	
calves		72			
	residual	72	ms_R	ξ_2	
	Total	80			

- In order to test hypothesis $H_0 : \mu_A = \mu_B = \mu_C = \mu_D$ must assume multivariate Normal distribution, so

$$\boldsymbol{y} \sim N\left(\boldsymbol{\mu} \otimes \mathbf{1}_k, \;\; \xi_0\boldsymbol{P}_0 + \xi_1\boldsymbol{P}_1 + \xi_2\boldsymbol{P}_2\right)$$

- Then test H_0 by comparing the VR with an F-distribution on $(3, 4)$ df

Calf feeding experiment
ANOVA and Linear Mixed Model

- ANOVA model assuming multivariate Normal distribution:

$$\boldsymbol{y} \sim N\left(\boldsymbol{\mu} \otimes \mathbf{1}_k,\ \ \xi_0 \boldsymbol{P}_0 + \xi_1 \boldsymbol{P}_1 + \xi_2 \boldsymbol{P}_2\right)$$

- Except that must set $\xi_0 = \xi_1$ so

$$\begin{aligned} \boldsymbol{y} &\sim N \quad \left(\boldsymbol{\mu} \otimes \mathbf{1}_k,\ \ \xi_1 \left(\boldsymbol{P}_0 + \boldsymbol{P}_1\right) + \xi_2 \boldsymbol{P}_2\right) \\ &\sim N \quad \left(\boldsymbol{\mu} \otimes \mathbf{1}_k,\ \ \xi_1 \boldsymbol{I}_m \otimes \boldsymbol{J}_k/k + \xi_2 \boldsymbol{I}_m \otimes \left(\boldsymbol{I}_k - \boldsymbol{J}_k/k\right)\right) \end{aligned}$$

- We can fit this as a linear mixed model

Linear Mixed Model

- The linear mixed model (LMM) for the data vector $\boldsymbol{y}$ is

$$\boldsymbol{y} = \boldsymbol{X}\boldsymbol{\tau} + \boldsymbol{Z}\boldsymbol{u} + \boldsymbol{e}$$

  - $\boldsymbol{\tau}$ is the vector of fixed effects with associated design matrix $\boldsymbol{X}$ (assumed full column rank)
  - $\boldsymbol{u}$ is the vector of random effects with associated design matrix $\boldsymbol{Z}$
  - $\boldsymbol{e}$ is the vector of residuals
- Variance models given by:

$$\text{var}\left(\boldsymbol{u}\right) = \boldsymbol{G} \quad \& \quad \text{var}\left(\boldsymbol{e}\right) = \boldsymbol{R}$$
$$\text{var}\left(\boldsymbol{y}\right) = \boldsymbol{Z}\boldsymbol{G}\boldsymbol{Z}^{\top} + \boldsymbol{R}$$

- Fitting the LMM $\Rightarrow$
  - Residual Maximum Likelihood (REML) estimates of variance parameters
  - Empirical Best Linear Unbiased Estimates (EBLUEs) of fixed effects
  - Empirical Best Linear Unbiased Predictions (EBLUPs) of random effects

Calf feeding experiment

Equivalence of ANOVA and Linear Mixed Model

- $\boldsymbol{\tau}$ is the $t-$ vector of fixed effects (overall mean and feed treatment effects) with associated design matrix $\boldsymbol{X}$ so that

$$\begin{aligned} \mathrm{E}(\boldsymbol{y}) &= \boldsymbol{X}\boldsymbol{\tau} \\ &\equiv \boldsymbol{\mu} \otimes \mathbf{1}_k \end{aligned}$$

- $\boldsymbol{u}$ is the $m-$ vector of random pen effects with associated design matrix $\boldsymbol{Z} = \boldsymbol{I}_m \otimes \mathbf{1}_k$
- Variance models given by:

$$\begin{aligned} \mathrm{var}(\boldsymbol{u}) &= \sigma_p^2 \boldsymbol{I}_m \;\; \& \;\; \mathrm{var}(\boldsymbol{e}) = \sigma^2 \boldsymbol{I}_{mk} \\ \mathrm{var}(\boldsymbol{y}) &= \sigma_p^2 \boldsymbol{I}_m \otimes \boldsymbol{J}_k + \sigma^2 \boldsymbol{I}_m \otimes \boldsymbol{I}_k \\ &\equiv \xi_1 \boldsymbol{I}_m \otimes \boldsymbol{J}_k/k + \xi_2 \boldsymbol{I}_m \otimes (\boldsymbol{I}_k - \boldsymbol{J}_k/k) \end{aligned}$$

where $\xi_1 = k\sigma_p^2 + \sigma^2$ and $\xi_2 = \sigma^2$

Calf feeding experiment

Equivalence of ANOVA and Linear Mixed Model

- Variance parameter estimates:

ANOVA	LMM	note/proviso
$\hat{\xi}_1,\ \hat{\xi}_2$	$\hat{\sigma}^2_p,\ \hat{\sigma}^2$ $\hat{\xi}_1 = k\hat{\sigma}^2_p + \hat{\sigma}^2$ $\hat{\xi}_2 = \hat{\sigma}^2$	allow $\hat{\sigma}^2_p < 0$

- Treatment effect estimates and inference:

ANOVA	LMM	note/proviso
$\hat{\mu}_i,\quad i{=}A,B,C,D$ se$(\hat{\mu}_i - \hat{\mu}_j)$ F test, df	$\hat{\mu}_i,\quad i{=}A,B,C,D$ se$(\hat{\mu}_i - \hat{\mu}_j)$ Wald test, df	se$(\hat{\mu}_i)$ not valid (ξ_0 not estimable) allow $\hat{\sigma}^2_p < 0$; use Kenward & Roger (1997) for Wald df

- How can we derive an appropriate LMM for a comparative experiment?
- We use an approach that we have called “Design Tableau”
- It can be used for quite complex non-orthogonal experiments, with the aim that it reproduces an ANOVA in orthogonal cases
- Design Tableau requires some definitions . . .

Comparative experiments

Some key definitions (Bailey, 2008)

- An *experimental unit* is the smallest unit to which a treatment can be applied
- A *treatment* is the entire description of what can be applied to an experimental unit
- An *observational unit* is the smallest unit on which a response will be measured. It is often called a *plot*.

Comparative experiments

Components of the design (Bailey, 2008)

- All designs have three components:
  - A plot structure: meaningful ways of dividing up the set of all plots
  - A treatment structure: meaningful ways of dividing up the set of all treatments
  - A design function: manner in which treatments are allocated to plots
- Plot and treatment structures can be described using *factors*

Comparative experiments
Design Tableau

Step 0 Talk to researcher and draw a picture of the experimental layout!

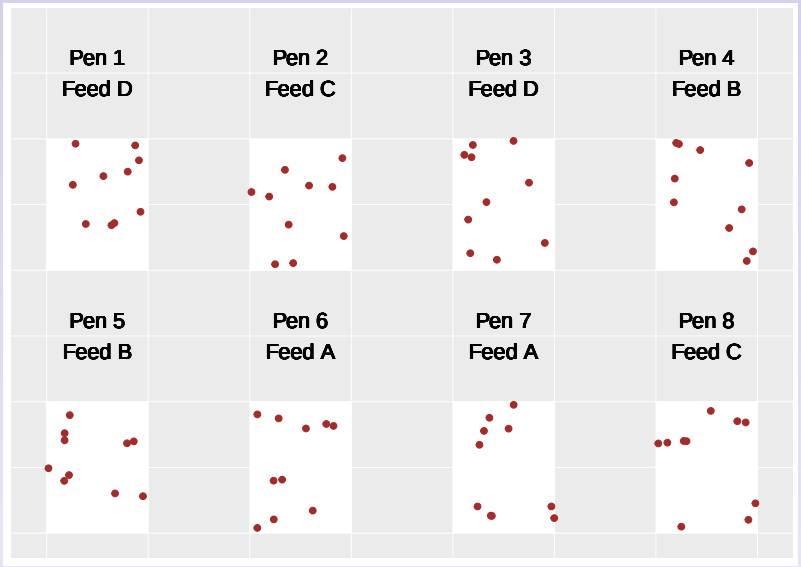
Step 1 Define treatments and list treatment factors

Step 2 Define plots (observational units) and list plot factors.

Step 3 Describe design function (how treatments are allocated to plots) and thence define experimental unit

Calf feeding example

Step 0



Step 1

- treatments = feeds (4 treatments)
- treatment factors = { Feed (4 levels) }

Step 2

- plots (observational units) = calves (80 units)
- plot factors = { Pen (8 levels), Calf (10 levels) }

Step 3

- design function: feeds allocated to calves in such a way that all 10 calves within a pen receive the same feed
- experimental units = pens

Step 4 Use factors from Step 1 to construct model formula for treatment structure (Wilkinson and Rogers, 1973, notation)

Step 5 Use factors from Step 2 to construct model formula for plot structure. Combinations of levels of factors in model formula must completely index observational units.

Step 6 Construct a table (Design Tableau) listing all terms in the treatment and plot model formulae from Steps 4 and 5. Indicate whether terms will be fitted in the LMM as fixed (treatment structure) or random (plot structure).

Step 7 Fit LMM commensurate with the Design Tableau

Calf feeding example

Design Tableau

Step 4 treatment structure model formula:
1 + Feed

Step 5 plot structure model formula:
Pen/Calf = Pen + Pen:Calf

Step 6 Design Tableau

Source	Term in model	Fixed or Random	Variance model
Mean	1	F	
Feed	Feed	F	
Pen	Pen	R	$\sigma_p^2 \boldsymbol{I}_m$
Pen:Calf	Pen:Calf (= residual)	R	$\sigma^2 \boldsymbol{I}_{mk}$

Calf feeding example

Step 7 using ASReml-R (Butler et al, 2009)

- Fit linear mixed model:
  calf.asr <- asreml(y $\sim$ 1 + Feed, random = $\sim$ Pen, residual = $\sim$ units, data= ...)
  - **1 + Feed**: fixed model formula, includes overall mean **1** by default
  - **random = $\sim$ Pen**: random model formula, default IID variance model, default constrained positive
  - **residual = $\sim$ units**: residual model formula, default IID variance model for **units** (factor with n levels)
- Estimates, $\hat{\mu}_i$, and sed for feed means:
  predict(calf.asr, classify="Feed")
- Test hypothesis $H_0 : \mu_A = \mu_B = \mu_C = \mu_D$
  Wald(calf.asr, denDF="algebraic")

Multi-phase comparative experiments

Milling yield example

- McIntyre (1955) first introduced concept of a two-phase experiment in which there are two separate randomisations (conducted at different times) and two different sets of experimental units
- Extension to more than two phases obvious
- Two-phase example: an experiment to compare wheat varieties in terms of milling yield (amount of flour)
- Varieties are first grown in a field experiment (Phase I) to produce bags of grain that are then taken to a laboratory to be milled (Phase II) to obtain the variable of interest

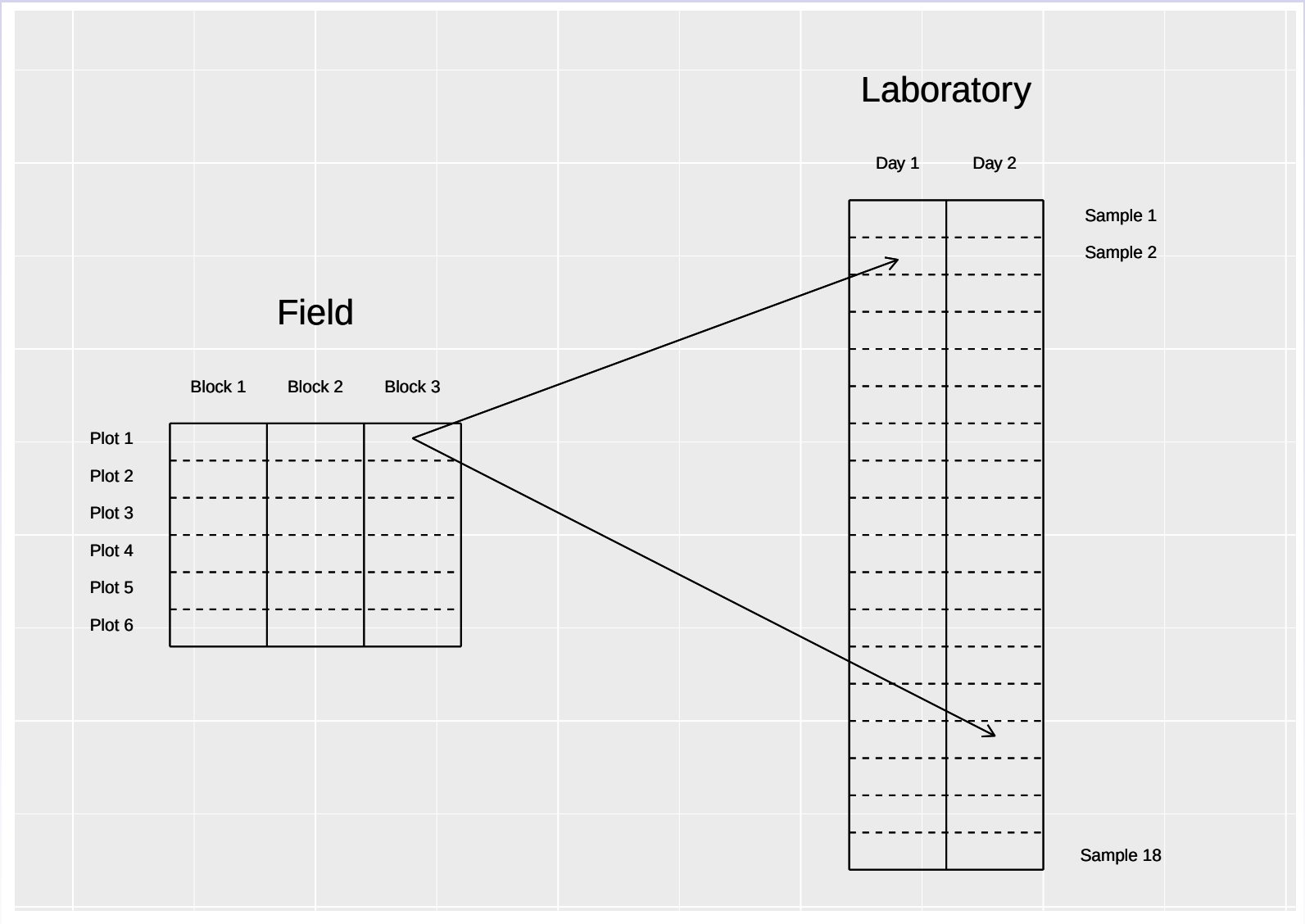
Multi-phase comparative experiments

Milling yield example

- Phase I field experiment:
  - $p = 18$ field plots divided into $r = 3$ blocks
  - A randomised complete block (RCB) design with plots within blocks allocated one of $t = 6$ varieties (A, B, C, D, E, F)
- Phase II milling experiment:
  - $n = 36$ samples in the milling process, divided into $d = 2$ days
  - An RCB design with samples within days allocated (grain from) one of the $p = 18$ plots from the first phase

Multi-phase comparative experiments

Milling yield example



Milling experiment: ANOVA table

Stratum	Source	df	ms	VR
mean		1		
	Mean	1		
	residual	0		
days		1		
blocks		2		
plots		15		
	Variety	5	ms_V	ms_V/ms_P
	residual	10	ms_P	
samples	residual	17		
	Total	36		

- Using Nelder (1965b) can show that information on
  - Mean entirely in mean stratum
  - Variety contrasts entirely in plots stratum
- Test hypothesis $H_0 : \mu_A = \mu_B = \mu_C = \mu_D = \mu_E = \mu_F$ by comparing the VR with an F-distribution on $(5, 10)$ df

Step 0 Talk to researcher and draw a picture of the experimental layout!

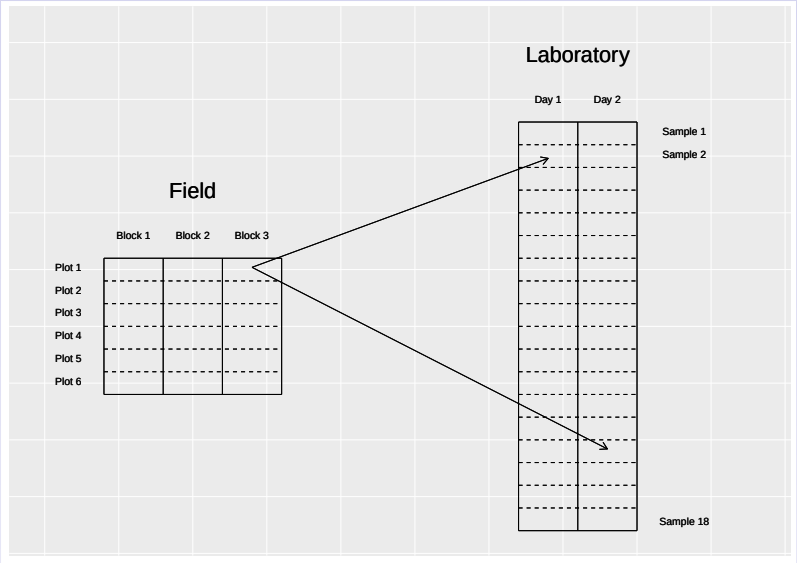
For each phase:

- **Step 1** Define treatments and associated factors
- **Step 2** Define plots (observational units) and factors
- **Step 3** Describe design function
- **Step 4** Model formula for treatment structure
- **Step 5** Model formula for plot structure
- **Step 6** Construct Design Tableau

Step 7 Fit LMM commensurate with the Design Tableau from the final phase

Milling example: Phase I

Step 0



Step 1
- treatments = varieties (6)
- treatment factors = { Variety (6 levels) }

Step 2
- plots (observational units) = plots (18 units)
- plot factors = { Block (3 levels), Plot (6 levels) }

Step 3
- design function: varieties allocated to plots within blocks

Milling example: Phase I

Step 4 treatment structure model formula:
1 + Variety

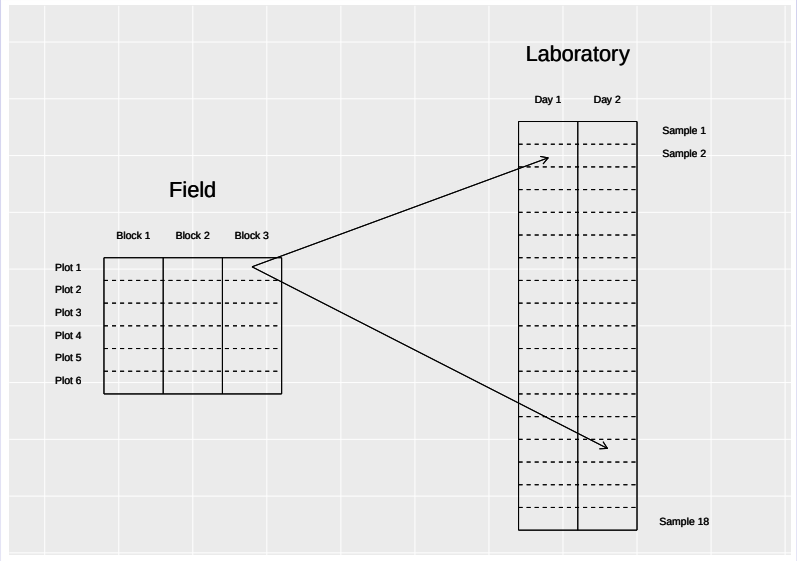
Step 5 plot structure model formula:
Block/Plot = Block + Block:Plot

Step 6 Design Tableau

Source	Term in model	Fixed or Random
Mean	1	F
Variety	Variety	F
Block	Block	R
Block:Plot	Block:Plot	R

Milling example: Phase II

Step 0



Step 1

- treatments = field plots (18)
- treatment factors = { Variety, Block, Plot }

Step 2

- plots (observational units) = samples (36 units)
- plot factors = { Day (2 levels), Sample (18 levels) }

Step 3

- design function: field plots allocated to samples within days

Milling example: Phase II

Step 4 treatment structure model formula:
1 + Variety + Block/Plot

Note that

- For Phase $s > 1$ this is given by the sum of treatment *and* plot models from phases $1 \ldots (s-1)$
- Fixed/Random status of terms maintained from previous phases
- Can add factors if new treatments applied

Step 5 plot structure model formula:
Day/Sample = Day + Day:Sample

Milling example

Step 6 Design Tableau from phase II

Source	Term in model	Fixed or Random	Variance model
Mean	1	F	
Variety	Variety	F	
Block	Block	R	$\sigma_b^2 \boldsymbol{I}_3$
Block:Plot	Block:Plot	R	$\sigma_p^2 \boldsymbol{I}_{18}$
Day	Day	R	$\sigma_d^2 \boldsymbol{I}_2$
Day:Order	Day:Order (=residual)	R	$\sigma^2 \boldsymbol{I}_{36}$

Step 7 fit linear mixed model using ASReml-R (Butler et al, 2009)

mill.asr <- asreml(y $\sim$ 1 + Variety,
random = $\sim$ Block + Block:Plot + Day,
residual = $\sim$ units, data= ...)

Design Tableau for comparative experiments

Summary: orthogonal designs

- Have demonstrated how Design Tableau can be used to derive a LMM that is a surrogate for randomisation-based ANOVA for experiments with orthogonal designs.
- Some provisos . . .
  - Allow negative estimates of variance components so can reproduce strata for valid inference
  - Use Kenward & Roger (1997) df adjustments so can use correct reference distribution for F-tests

Design Tableau for comparative experiments

Summary: non-orthogonal designs

- Very few of the experiments we analyse use orthogonal designs!
- Also typically complex (unbalanced multi-environment trials; longitudinal data; multi-phase experiments with composite sampling . . .)
- But we always start with Design Tableau to obtain the terms that reflect the randomisation used in the experiment. This provides safe-guard against false replication, omission of strata, . . .
- For most experiments, Design Tableau provides base-line "working model" which we may extend in various ways eg. incorporate spatial correlation models for field trials, factor analytic models for variety by environment effects, . . .

Example: non-orthogonal design

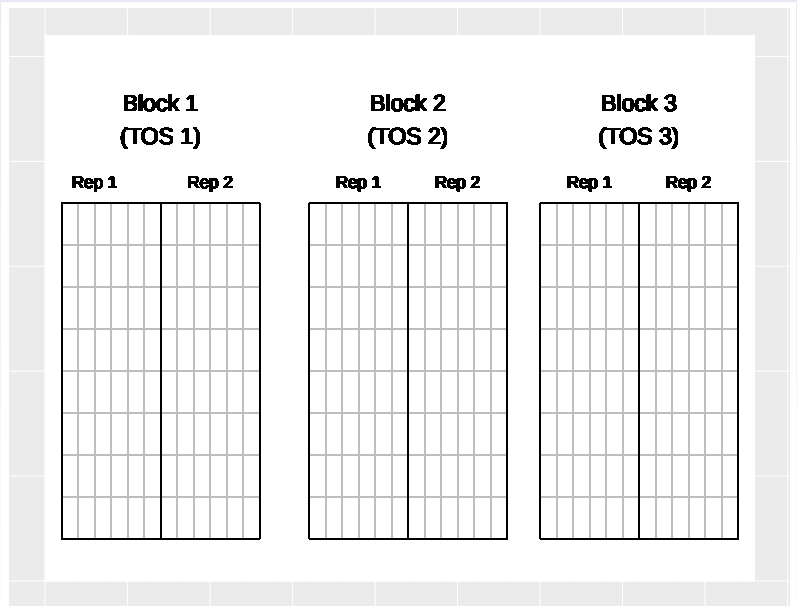
Frost expression experiments

- Frost damage a key issue for Australian wheat growers
- Frost expression experiments (FEEs) conducted at sites across Australia to provide information for growers on tolerance of commercial and near release varieties
- FEEs are field trials in which varieties exposed to natural frost events
- Variable of interest, frost induced sterility (FIS), obtained after frost events: ratio of number of sterile grains to total grains for individual tillers

Frost expression experiments

Protocol for single FEE

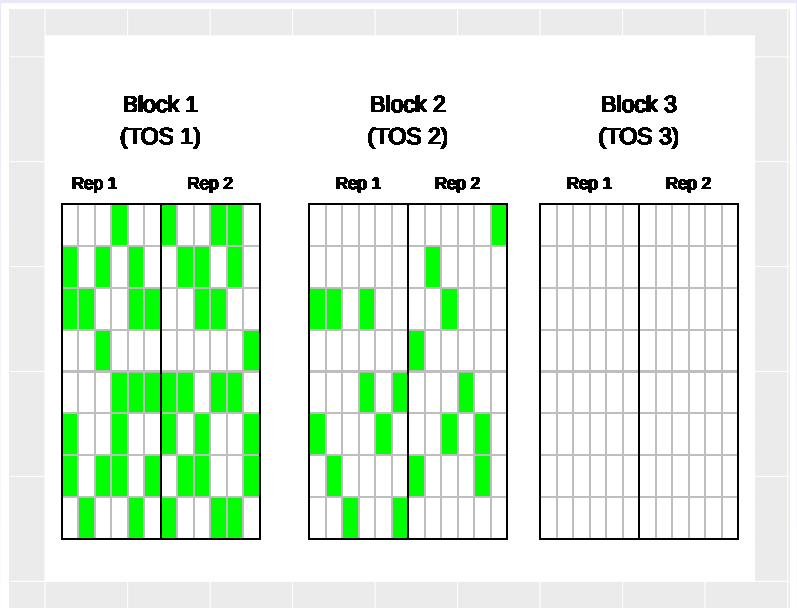
- Each variety grown at several times of sowing (TOS) to ensure some tillers at correct stage of development (SOD) when frost event occurs
- All plots for a TOS grouped together in a block
- Within each TOS block a randomised complete block design for varieties
- An illustrative example: 3 TOS blocks; 48 varieties; 2 replicates of varieties within each TOS block



Frost expression experiments

Protocol for single FEE

- After a frost event, researchers walk through the trial
  - Visually assses if any tillers in a plot are at an SOD of interest (flowering and ear peep)
  - If so, tag these tillers (up to a maximum of 30 per stage per plot), but leave the plant to continue growing
  - About 2 weeks after frost event, tagged tillers are cut and individually bagged; grains counted to provide FIS
  - Highly unbalanced: only a subset of plots measured for a single frost event (and number varies between TOS blocks); number of tillers measured in a plot varies between plots



Frost expression experiments

Some key issues

- Data for single frost event highly unbalanced
- Typically multiple frost events so potential for repeated measurements on a plot. Even more imbalance (number of repeated measurements per plot varies and may be 0)
- Aim is to assess variety tolerance but expect variety by TOS (careful!), variety by SOD and possibly variety by TOS by SOD interactions
- Finally there are 11 FEEs so a multi-environment trial analysis required to examine interactions with environment
- Where to begin?

Frost expression experiments

Impact of Design Tableau

- Where to begin?
  - Start with Design Tableau for a single trial and frost event
  - Then Design Tableau for a single trial and multiple frost events
  - Then Design Tableau for multiple trials and frost events
  - Finally modify LMM with complex variance models to accommodate multi-environment and longitudinal aspects, but keep Design Tableau at the core
- Have successfully used Design Tableau to determine an appropriate LMM for analysis of FEE (See Cocks and Cullis, in preparation)
- A great result for industry:
  - previous analyses of these data did not use our approach and failed to identify key issues; industry lost faith in results
  - with use of Design Tableau and close association with researchers we have regained industry and grower confidence in the results. Complete acceptance.

Design Tableau for comparative experiments

Summary: model based analysis

- Design Tableau is an implicit component of our paradigm not only for analysis but also for generation of designs
- We generate model based designs using OD (Optimal Design, Butler, 2013) software within the R environment. Crucial for multi-phase designs (eg. in example, treatments in phase II are *plots* but use OD to optimise for *variety* effects)

Design Tableau for comparative experiments

Key references

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- Nelder, J.A. 1954. *The Interpretation of negative components of variance*. Biometrika
- Nelder, J.A. 1965a. *The analysis of randomized experiments with orthogonal block structure. I. Block structure and the null analysis of variance*. Proceedings of the Royal Society, A.
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