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PRACTICAL INFORMATION

Experimental design in this course:

- brief overview of ideas and methods,
- more focus on data analysis than on design construction,
- more detailed references — textbook extras, and
 - * Cochran & Cox: Experimental Designs (UPEI library),
 - * Box, Hunter & Hunter: Statistics for Experimenters (UPEI lib.),
 - * Dean & Voss: Design and Analysis of Experiments¹.

Today's lecture:

- follow-up from lab?
- principles of experimental design,
- a range of classical block designs.

Guidelines for textbook reading:

- skip entirely: discussions of efficiency (13.2.3, 13.3.5, pp. 362₇–368¹¹) and advanced sections (13.5, 14.3-7),
- skip for now: refs to Ch. 11-12, incl. Hasse diagrams,
- postpone (Session 9): cross-over designs (13.3.1, 13.3.6).

Other news:

- home assignments: #3 due on Monday (4/3),
- definitely time to make progress on your project.

¹ Available through UPEI library e-book collections; link via course homepage.

INTRODUCTION TO EXPERIMENTAL DESIGN

What characterises an experiment?²

the experimenter imposes *treatments* onto the *experimental units* from which *responses* are measured.

Advantages of an experiment:

- allows (potentially) to conclude about causation,³
- can be designed to give small error in comparisons,
- can be designed to avoid/minimise bias in comparisons.

Plain glossary for experimental design:

- treatments: the different procedures to compare,
- experimental units: the “things/subjects/individuals” to which treatments are applied,
- responses: outcomes of interest to compare, observed at the units after treatments are applied,
- randomisation: random assignment of treatments to experimental units (to avoid unexpected patterns),
- experimental error: variation between equally treated units (both population and observed values),

² As opposed to observational studies.

³ The reasoning goes: “If experimental units differ only (ideally) by their treatments, any differences beyond random fluctuations in their responses must be caused by the treatments”.

- measurement units: the actual objects on which responses are measured (may be smaller than the experimental units),
- blinding: response evaluators do not know the treatment allocation,
- control: treatment consisting of “no treatment”, possibly *placebo* (inactive treatment),⁴
- factor: (controlled) explanatory, categorical variable,
- level/category: specific value of factor/treatment,
- replication: multiple “identical” experimental units (sometimes, confusingly, repetitions of the entire experiment⁵),⁶
- blocking: division of experimental units into homogeneous groups (in order to reduce experimental error),
- balancedness
 - = all treatment levels occur the same number of times,
 - * *desirable* property of a design, but *not indispensable*,
 - * gives same precision on all treatment estimates and comparisons, and simple computational formulae,
 - * necessary for some ANOVA methods — however, (general) linear model methods always apply,
- (technical) confounding: the effect of one factor/treatment cannot be distinguished from that of another factor/treatment (different meaning than in epidemiology!).

⁴ The main purpose of a control group is to control lurking variables/confounders.

⁵ Repetition of an entire experiment would usually be considered a blocking factor.

⁶ The purpose of replication is to enable estimation of experimental error.

A CHECKLIST FOR PLANNING EXPERIMENTS

(from Dean & Voss, Chapter 2, pages 7–14)⁷

- (a) Define the objectives of the experiment.
- (b) Identify all sources of variation, including:
 - (i) treatment factors and their levels,
 - (ii) experimental units,
 - (iii) blocking factors, noise factors, and covariates.
- (c) Choose a rule for assigning the experimental units to the treatments (the narrow meaning of experimental design).
- (d) Specify the measurements to be made, the experimental procedure, and the anticipated difficulties.
- (e) Run a pilot experiment.
- (f) Specify the (statistical) model.
- (g) Outline the (statistical) analysis.
- (h) Calculate the number of observations that need to be taken.
- (i) Review the above decisions. Revise if necessary.

⁷ Chapter 2 is also available at the VHM 802 Moodle account.

COMPLETELY RANDOMISED DESIGN

In a completely randomised design, all treatments are allocated at random among the experimental units (using a method for random selection). In all other respects, the units are treated as equally as possible.

⇒ (idea/rationale:)

Differences in response must be due to either treatment effects or play of chance in random assignment of units.

Comments:

- + simple/easy to understand, carry out and analyse,
- + flexible (allows any number of levels and replications),
- all experimental units need to be “homogeneous”, otherwise the random variation will be large,
- if a good grouping of experimental units exists (either in their state before treatments are applied or in general conditions during the experiment), other designs will be more efficient (give greater precision),
- primarily for small designs with no obvious grouping,
- completely randomised designs with a large number of replications (and DF_E) are “suspect” (rarely realistic).

Randomisation may be achieved by randomly reordering experimental units (easy in statistical software).

(RANDOMISED) BLOCK DESIGN

Blocks = groups of homogeneous experimental units, i.e., units are more alike within than between groups, before and during experiment.

In a randomised block design, treatments are assigned randomly to the units within each block; typically, each treatment occurs once (≥ 1 : *complete* design) per block.
 \Rightarrow (idea/rationale:)

More accurate to compare similar units (in same block) and to aggregate such comparisons across blocks.

Examples of factors used to form blocks:

- agriculture: areas in fields,
- animal science: litters, groups (age, weight, sex), environment (herd),
- human medicine: twins (matched pairs design), family, groups (as above + health, social status, lifestyle...),
- general: time, operator (surgeon, technician).

Comments on block designs:

- + improvement in accuracy if efficient groups available,
- +/- minor added complexity in design and analysis,
- less flexible (block size should match no. treatments),

Randomisation: by randomly reordering experimental units within each block.

STATISTICAL ANALYSIS OF BLOCK DESIGNS

Statistical model for observations (y_{ij}) on treatments ($i = 1, \dots, g$) in blocks ($j = 1, \dots, r$):

$$y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij},$$

where

- α_i and $\beta_j \sim$ effects of treatment i and block j , resp.,
- the errors $\varepsilon_{11}, \dots, \varepsilon_{gr}$ are i.i.d. and $\sim N(0, \sigma^2)$.
- block effects are (usually) additive to treatments:
 - * assumes that tx effects do not depend on blocks,
 - * most useful when tx by block interaction believed to be absent or weak,⁸
 - * non-additive block effects: only possible with replicated treatments within blocks or a factorial treatment structure (and omitted interactions).

Statistical analysis follows usual ANOVA/linear model principles, except that

- block effects often of less interest (for interpretation),
- blocks are usually not randomized \Rightarrow tests for block effects could be omitted entirely (GO),
- (technical) possible to compute *efficiency* of block design relative to compl. rand. design (“did the block design work?”), see GO for details (not part of course).

⁸ Ignoring an existing tx by block interaction \Rightarrow inflated σ^2 and loss of power.

(UNREPLICATED) BLOCK DESIGN – EXAMPLE

Mealybug example: (GO Example 13.1)

- changes (before – after treatment) in counts of mealybugs,
- 3 treatments (water, spores, oil), 5 plants (~ blocks),
- experimental unit: branches of cycad plants,
- measurement units: two patches per branch treated the same way,

- data layout:

Treatment \ Plant	1	2	3	4	5
water	-9	18	10	9	-6
	-6	5	9	0	13
spores	-4	29	4	-2	11
	7	10	-1	6	-1
oil	4	29	14	14	7
	11	36	16	18	15

- outcome: average change across two patches per branch.

ANOVA table for mealybug data:

Source	DF	SS	MS	<i>F</i>	<i>P</i> -value
Treatments	2	432.0	216.0	12.2	.004
Plants	4	686.4	171.6	(9.7)	(.004)
Error	8	141.8	17.7		
Total	14	1260.2			

- * clear treatments effect: oil treatment has larger change than the others: water $(4.3)^b$, spores $(5.9)^b$, oil $(16.4)^a$,
- * plant effects also clearly significant (but less important).

LATIN SQUARE DESIGN

- definition: design with
 - g treatments,
 - two blocking variables,
 - g^2 experimental units arranged in a square,
 and every treatment exactly once in each row / column,
- examples: (*cyclic* Latin squares, plus permutations)

$$g = 2: \begin{array}{|c|c|} \hline A & B \\ \hline B & A \\ \hline \end{array} \quad \begin{array}{|c|c|} \hline B & A \\ \hline A & B \\ \hline \end{array}$$

$$g = 4: \begin{array}{|c|c|c|c|} \hline A & B & C & D \\ \hline B & C & D & A \\ \hline C & D & A & B \\ \hline D & A & B & C \\ \hline \end{array}$$

$$g = 3: \begin{array}{|c|c|c|} \hline A & B & C \\ \hline B & C & A \\ \hline C & A & B \\ \hline \end{array} \quad \begin{array}{|c|c|c|} \hline A & C & B \\ \hline C & B & A \\ \hline B & A & C \\ \hline \end{array}$$

- advantage: takes into account two blockings
 \Rightarrow potentially more accurate (lower exper. error),
- disadvantage: specific requirements for dimensions of design (no. of blocks, no. of experimental units),
- randomisation between squares: not easy to do properly⁹; a simple and “crude” method randomly permutes rows, columns and symbols,
- modelling: additive effects of rows and columns,
- balanced in the treatments: “nice”, simple analysis.

⁹ Fisher-Yates procedure based on *standard* squares tabled, see GO p. 327.

LATIN SQUARE DATA EXAMPLE

Mangold (wurzel) example: (Mercer & Hall 1911; field trial)

- yield of plots located in a 5×5 Latin square and with 5 (unspecified) treatments,

y_{ijk} = weight of mangold in plot with
treatment i at location (j, k) ,

$i = A, B, C, D, E \sim$ treatments,

$j, k = 1, 2, 3, 4, 5 \sim$ rows and columns,

technical note: only some sets (i, j, k) occur,

- data layout: (treatments and yields)

D 376	E 371	C 355	B 356	A 335
B 316	D 338	E 336	A 356	C 332
C 326	A 326	B 335	D 343	E 330
E 317	B 343	A 330	C 327	D 336
A 321	C 332	D 317	E 318	B 306

- statistical model (additive treatments and blocks):

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ijk},$$

where the ε_{ijk} 's are i.i.d. and $\sim N(0, \sigma^2)$.

Extension of design to allow for two treatments: Graeco-Latin squares (Section 13.4).

MANGOLD DATA RESULTS

ANOVA table for additive model:

Source	DF	SS	MS	F	P -value
Rows	4	4240.2	1060.1	(7.25)	(.003)
Columns	4	701.8	175.5	(1.20)	(.36)
Treatments	4	330.2	82.6	0.56	.69
Error	12	1754.3	146.2		
Total	24	7026.6			

- no indication of treatment effects, but D highest; contrast comparing D to average of others:

$$\begin{aligned}\hat{w} &= 4\hat{\alpha}_D - \hat{\alpha}_A - \hat{\alpha}_B - \hat{\alpha}_C - \hat{\alpha}_E \\ &= 4\bar{y}_{D..} - \bar{y}_{A..} - \bar{y}_{B..} - \bar{y}_{C..} - \bar{y}_{E..} = 34.4, \\ \text{SE}(\hat{w}) &= \sqrt{\text{MS}_E (4^2 + 1^2 + 1^2 + 1^2 + 1^2)/5} = 24.18, \\ t &= \hat{w}/\text{SE}(\hat{w}) = 34.4/24.2 = 1.42, \\ \text{SS}(\hat{w}) &= t^2 \text{MS}_E = 295.84,\end{aligned}$$

- * accounts for 90% (295.84/330.24) of variation,
- * clearly non-significant even if pre-planned,
- no column effects, but clear row effects: row 1 > rows 2–4 > row 5 (maybe not particularly interesting).

Further analyses:

- diagnostics: all look good,
- model reduction: possible to drop non-significant effects, but not necessary.

INCOMPLETE BLOCK DESIGNS

Incomplete: the blocks do not comprise all treatments (combinations of treatment factors).

Why of interest?

- maybe limited block sizes (e.g., litters or twins),
- maybe save experimental units (economy),
- missing values \Rightarrow incomplete blocks.

Main points about incomplete block designs:

- unbalanced (in an extreme form) \Rightarrow order of testing for factors is important: partial SS \neq sequential SS,¹⁰
- “balanced” incomplete block design (BIBD): balanced in the sense that all pairs of treatments occur equally often in the same block \Rightarrow same precision on all treatment comparisons,
- many specialised designs exist with certain properties (GO Sections 14.3-7; not part of course),
- statistical analysis: using methods for (general) linear models, in particular *least squares means*¹¹.

¹⁰ Recall that partial/adjusted SS \sim removing effect while keeping all others, and sequential SS \sim removing effects sequentially (bottom up).

¹¹ Recall that least squares means are adjusted for other factors by giving their levels/categories equal weights; this would seem the correct approach when unequal representation occurs by design.

BALANCED INCOMPLETE BLOCK DESIGN (BIBD)

A balanced incomplete block design with g treatments and b blocks must meet the requirements,

- all treatments occur the same no. of times, r ,
- all blocks are of the same size, k ,
- every pair of treatments “meet” the same no. of times, λ ,

Then the following relations hold:

$$rg = bk \quad \text{and} \quad \lambda(g - 1) = r(k - 1).$$

Construction of BIBD's:

- designs do not always exist (even if relations satisfied),
- designs tabled in textbooks, e.g. GO or Cochran & Cox.

Statistical model has additive treatments and blocks:

$$y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij},$$

where $i = 1, \dots, g \sim$ treatments and $j = 1, \dots, b \sim$ blocks, and not all pairs (i, j) occur. Alternative model formulation:

$$y_i = \mu + \alpha_{\text{treat}(i)} + \beta_{\text{block}(i)} + \varepsilon_i.$$

Technical notes on analysis:

- treatment SE computable by hand, using “effective sample size” ($\lambda g/k$) instead of r .
- “intra-block” analysis \sim usual linear model, and
“inter-block” analysis \sim random effects model (Session 9).

BIBD EXAMPLES

Simplest BIBD ($g=3, b=3, r=2, k=2, \lambda=1$):

Block	1	2	3
Treat-	A	B	C
ment	B	C	A

Dish detergent example: (GO Example 14.2)

- count no. of plates before foam disappears, in “sessions” with 3 simultaneous operators and 3 basins,

y_{ij} = no. of plates for detergent i in session j

$i = A, B, C, D, E, F, G, H, J \sim$ detergents, treatments

$j = 1, \dots, 12 \sim$ sessions, blocks,

- experimental layout:

Block	1	2	3	4	5	6	7	8	9	10	11	12
Treat-	A	D	G	A	B	C	A	B	C	A	B	C
ment	B	E	H	D	E	F	E	F	D	F	D	E
	C	F	J	G	H	J	J	G	H	H	J	G

- BIBD with $g=9, b=12, r=4, k=3, \lambda=1$,
- further treatment structure:
 - * A,B,C,D \sim detergent I + doses 3,2,1,0 of additive,
 - * E,F,G,H \sim detergent II + doses 3,2,1,0 of additive,
 - * J \sim control.

YOUDEN SQUARE

- incomplete block design with two blocking factors, e.g. Mangold data with last column omitted:

D 376	E 371	C 355	B 356	(A .)
B 316	D 338	E 336	A 356	(C .)
C 326	A 326	B 335	D 343	(E .)
E 317	B 343	A 330	C 327	(D .)
A 321	C 332	D 317	E 318	(B .)

- design properties:¹²
 - * first blocking factor (“rows”) and treatments form a BIBD (example: $g=5$, $b=5$, $r=4$, $k=4$, $\lambda=3$),
 - * second blocking factor (“columns”) has every treatment once per block (\sim complete and balanced blocks),
- advantages: retains “nice” analysis for treatments from BIBD while allowing for two blocking factors,
- note: a Youden square is *not* a square so name probably originates from relation to Latin squares (dropping one row or one column).

¹² These two properties may also be taken as the definition of more general Youden squares, see GO Section 14.2.