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Practical Information

Today's lecture:

- cross-over designs: based on lecture notes + references:
  - GO Sections 13.3.1, 13.3.4, 13.3.6, 16.7,
  - [1] Senn (2005), Crossover Designs, In: *Encyclopedia of Biostatistics* (available electronically at UPEI library),
  - [2] Toutenburg (2002), *Statistical Analysis of Designed Experiments*, Chapter 9 (also electronic access at UPEI library),
- introduction to random effects models:
  - in both VHM 802 and VHM 802/812; different focus:
    - today: ANOVA-based analysis, balanced data, continuous outcome,
    - next week: split-plot or hierarchical design,
    - VHM 802/812: likelihood-based analysis, unbalanced data, continuous and discrete outcomes.
  - textbook reading:
    - Chapter 11 (skip Sections 4, 6 and 8-9),
    - supplementary notes[1] on linear mixed models (random effects models), part of course curriculum.

Other news:

- home assignment: 4 (due today),
- project outline due March 17 (Tuesday).

[1] The notes contain essentially the same topics as the textbook, but omit detailed formulae and calculations for EMS-based estimates and tests, which are beyond the core course curriculum.

Introduction to Cross-Over Designs

Definition of a cross-over trial:

> "a trial in which individual subjects are given sequences of treatments with the object of studying differences between individual treatments (or subsequences of treatments)" [1]

- idea: each subject forms a block (or one's own control)
  $\Rightarrow$ reduced variation (by eliminating between-subject variation),
- advantages:
  - (potentially) (much) higher efficiency than completely randomized design,
  - individual's reactions to treatments may be studied,
- drawbacks:
  - longer execution time $\Rightarrow$ logistical challenges, greater risk of subject dropouts,
  - more complex data analysis (due to added risk of bias caused by carry-over effects),
- simple 3×3 Latin square examples: Bioequivalence (GO Example 13.6), Rabbits (Additional exercise 7.2),
- a special case of repeated measures (Lecture 12).

Common terminology:

- periods: occasions where subjects are treated (and measured),
- carry-over effect: residual effect from one period to the next,
- wash-out period: non-treatment interval between periods to eliminate/reduce carry-over effects.

Simplest Design: AB/BA

- two treatments (A and B),
- two periods (1 and 2),
- two groups of subjects:

	period 1	period 2
subject group 1	A	B
subject group 2	B	A

Data example: Scents and learning[2] — can pleasant (floral) aromas help a student learn better?

- completion times for pencil and paper mazes for 21 subjects with scented (S) and unscented (U) masks,
- 11 subjects used unscented masks first, 10 subjects scented masks first (1 subject excluded):

subject	sequence	mean U	mean S	diff U−S	diff 1−2
1	US	30.6	38.0	-7.4	-7.4
2	SU	48.4	51.6	-3.2	3.2
3	US	60.8	56.7	4.1	4.1
4	SU	36.1	40.5	-4.4	4.4
5	US	68.5	49.0	19.5	19.5
6	SU	32.4	43.2	-10.8	10.8
...	...	...	...		

[2] Data available at: `lib.stat.cmu.edu/DASL/Datafiles/Scents.html`.

Analysis of AB/BA Design

Assuming no carry-over effects, simple approaches work:

- compare treatments by two-sample analysis (e.g. t-test) of sequences for differences $1-2$,[3]
- compare periods by two-sample analysis (e.g. t-test) of sequences for differences A$-$B.

Combined analysis of response y_{ijk} in period j for k'th subject with treatment sequence i, using the model

$$y_{ijk} = \mu_{ij} + s_{ik} + \varepsilon_{ijk}, \quad \text{where}$$

* μ_{ij} is the mean for sequence i in period j, given by:

sequence i	period 1 ($j=1$)	period 2 ($j=2$)
AB	$\mu + \alpha_A + \beta_1$	$\mu + \alpha_B + \beta_2 + \lambda_{AB}$
BA	$\mu + \alpha_B + \beta_1$	$\mu + \alpha_A + \beta_2 + \lambda_{BA}$

  - $\alpha_A, \alpha_B \sim$ treatment effects,
  - $\beta_1, \beta_2 \sim$ period effects,
  - $\lambda_{AB}, \lambda_{BA} \sim$ carry-over effects.
* s_{ik} is the effect of subject k with treatment sequence i,
* ε_{ijk} is the error term $\sim \mathrm{N}(0, \sigma^2)$,
* same inference as above if $\lambda_{AB} = \lambda_{BA} = 0$,
* complex analysis ([1],[2]) if λ's $\neq 0$.

[3] Treatment comparison by a paired two-sample analysis for measurements for A and B (effectively a one-sample analysis for differences A$-$B) is only valid when no period effects exist.

More Treatments and Periods

Examples of extensions of AB/BA design:

- 2 tx, > 2 periods: enables modelling of carry-over effects in analysis, e.g. for sequences AABB and BBAA:

	Sequence				Sequence			
carry-over model	A	A	B	B	B	B	A	A
"prev. period"	$-$	λ_A	λ_A	λ_B	$-$	λ_B	λ_B	λ_A
"change only"	$-$	0	λ_{AB}	0	$-$	0	λ_{BA}	0
"prev. + present"	$-$	λ_{AA}	λ_{AB}	λ_{BB}	$-$	λ_{BB}	λ_{BA}	λ_{AA}

- 3 tx, 3 periods: to ensure balancedness of tx's in periods traditionally laid out in Latin squares, e.g.,

A	B	C
B	C	A
C	A	B

A	C	B
C	B	A
B	A	C

  where rows $\sim$ periods, columns $\sim$ subjects, symbols $\sim$ tx,

  - simple analysis when no carry-over effects,
  - desirable to include all sequences by combining two different Latin squares (as shown above),

- g tx, g periods: use (multiple) $g \times g$ Latin squares,
- g tx, $k < g$ periods: use incomplete block design, preferably BIBD (g, b, k, r, λ) or Youden square.

Combining Latin Squares

Multiple Latin squares in same design:

- increases the error degrees of freedom ⇒ larger power,
- several options for modelling, dep. on data context.

Notation and Model:

y_{ijkl} = outcome for tx i in row j and column k in square l,

$$y_{ijkl} = \mu + \alpha_i + \beta_{j(l)} + \gamma_{k(l)} + \varepsilon_{ijkl},$$

- row ($\beta_{j(l)}$) and column ($\gamma_{k(l)}$) effects "nested in" (separate for) squares ∼ different effects across squares,[4]
- a (row or column) block effect may be assumed the same in all squares, e.g. period effects:
  - *same effects* if all subjects go through same periods,
  - *different effects* if periods are not the same (e.g. because of different ages of subjects),

2 modelling refinements:

- square type[5] interactions: carry-over effects may show up as interactions between square type and periods,
- residual effects may be modelled directly to split each tx effect into "direct" and "residual" effects.

[4] See 9L−14 for details about nesting, and examples for specification in software.
[5] The square type is determined by the carry-over combinations it contains.

Latin Square Cross-Over Trial Examples

Bioequivalence trial (GO Example 13.10) with 12 subjects in 4 Latin squares.

Milk production example $\sim$ cross-over trial (GO Example 13.12):

- milk yield of cows during three periods with different diets,

$$\begin{aligned} y_{ijkl} &= \text{yield for cow } k \text{ in square } l \text{ in period } j \text{ on diet } i \\ i &= \text{A,B,C} \sim \text{diets (roughage, limited grain, full grain)} \\ j &= 1,2,3 \sim \text{period (for each cow)}, \\ k &= 1,\ldots,3 \sim \text{cow number (within squares)} \\ l &= 1,\ldots,6 \sim \text{Latin square number.} \end{aligned}$$

	Cow			Cow			Cow		
Period	1	2	3	7	8	9	13	14	15
1	A	B	C	A	B	C	A	B	C
2	B	C	A	B	C	A	B	C	A
3	C	A	B	C	A	B	C	A	B

	Cow			Cow			Cow		
Period	4	5	6	10	11	12	16	17	18
1	A	B	C	A	B	C	A	B	C
2	C	A	B	C	A	B	C	A	B
3	B	C	A	B	C	A	B	C	A

- 6 separate Latin squares,
  - $*$ two types of Latin squares (top/bottom), 3 replicates of each,
  - $*$ top $\sim$ diet order AB, BC, CA; bottom $\sim$ AC, CB, BA,
- basic statistical model (additive, no square effects),

$$\begin{aligned} y_{ijkl} &= \mu + \alpha_i + \beta_j + \gamma_{k(l)} + \varepsilon_{ijkl}, \text{ or} \\ y_i &= \mu + \alpha_{\text{diet}(i)} + \beta_{\text{per}(i)} + \gamma_{\text{cow}(i)} + \varepsilon_i, \end{aligned}$$

where the errors are i.i.d. and $\sim \text{N}(0, \sigma^2)$.

Introduction to Random Effects Models

Fixed and random effects:[6]

- "fixed" effect: modelled by usual parameters (α's or β's: fixed, unknown constants $\sim$ factors or regressors),
- "random" effect: modelled by random variables.

Terminology and relationships:

- mixed
random effects
variance component } models $-$ the same,
- "mixed" $\sim$ containing both fixed and random effects,
- multi-level
hierarchical } models $-$ the same,
and special type of mixed models,
- variance components are mathematical constructs used in mixed models.

Motivations for random effects (decreasing importance):

- data structure, e.g. hierarchical (next lecture),
- correct analysis of explanatory variables allocated to larger experimental units ("split-plot" idea: next lecture),
- factor where interest is in variation between levels rather than specific levels in study:
  - levels may be randomly selected,
  - levels should represent "population",

  Examples (1-way/2-way ANOVA models): cartons, laboratories.

[6] Standard notation (but not GO!) uses Greek letters for fixed effect parameters and Latin letters for random effect variables.

Random Effects 1-Way ANOVA

Carton example: (GO Section 11.1, Example 11.2)

- strength of cardboard carton produced by 10 machines (restricted to 1 operator and 1 glue),

outcome	machine (i)						
y_{ij}	1	2	3	4	...	9	10
	157.97	160.28	156.81	156.26	...	149.88	153.33
	157.16	162.31	153.56	157.22	...	160.86	153.30

  notation: y_{ij} = strength of carton j from machine i,

- random effects 1-way ANOVA model:

$$y_{ij} = \mu + A_i + \varepsilon_{ij}, \quad i = 1, \ldots, a\,(10);\ j = 1, \ldots, n\,(2),$$

  where, as usual, the ε_{ij}'s are i.i.d. and $\sim \mathrm{N}(0, \sigma^2)$, and in addition the A_i's are i.i.d. and $\sim \mathrm{N}(0, \sigma_A^2)$,

- interpretations:
  - A_i = random effect of ith machine (in principle, assumed drawn from a suitable population of machines),
  - σ_A^2 = variability between machines (in that population),
  - random variation : *between machines* and *between cartons*,
  - $\mathrm{Var}(y_{ij}) = \sigma_A^2 + \sigma^2$ (sum of two *variance components*),

- new issues for statistical analysis:
  - estimation of σ_A^2: $\hat{\sigma}_A^2 = (\mathrm{MS}_A - \mathrm{MS}_E)/n$,
    (derived from the math. fact: $\mathrm{E}(\mathrm{MS}_A) = \sigma^2 + n\sigma_A^2$),
  - estimation of μ: $\hat{\mu} = \bar{y}_{\cdot\cdot}$, $\mathrm{SE}(\hat{\mu}) = \sqrt{\mathrm{MS}_A/(an)}$,
  - extra model check: normality of A_i's (using $\bar{y}_{i\cdot}$'s).

Two Datasets (from the Notes)

Ex. 1: Laboratory testing (Environmental Res. Inst., 1992)

- conc. of 4-methylphenol measured at different labs,
- labs selected among accredited labs for analysis,
- purpose: determine variation within and between labs,
- data for 5 labs, 3 dilutions and 2 samples:

phenol (μg/l)	Dilution					
Laboratory	1		2		3	
A	5.5	4.7	9.8	10.3	11.6	11.8
B	7.7	7.5	12.4	12.5	16.4	17.0
C	7.4	7.1	12.5	11.8	15.9	16.2
D	6.5	7.1	10.0	9.4	12.6	12.7
E	6.5	7.0	11.0	9.9	13.5	12.7

Ex. 2: Pig breeding (Snedecor & Cochran, 1967)

- weight gain for pigs bred from different sires and dams,
- data for 5 sires, 2 dams and 2 pigs:

weight gain	Sire				
Dam	1	2	3	4	5
1	2.77	2.28	2.36	2.87	2.74
	2.38	2.22	2.71	2.46	2.56
2	2.58	3.01	2.72	2.31	2.50
	2.94	2.61	2.74	2.24	2.48

Repeatability and Reproducibility

Fact: laboratory analyses do not give *same* result when replicated (that is, on identical samples).

Sources of variation (assuming same laboratory method):

- laboratory,
- technician / equipment,
- "day" (time),
- pure replication error.

Idea: compute statistic to summarize variation — the value *not* exceeded with probability 95% by the difference between two measurements taken:

- under identical conditions → repeatability r,
- under "similar" conditions → reproducibility R.

Lab example (dilution 1):

- concentration of phenol in identical samples submitted to 5 laboratories (A-E),
- notation: y_{ij} = conc. measured for jth sample at lab. i,
- model: $y_{ij} = \mu + A_i + \varepsilon_{ij}$, for i =A,B,C,D,E; j=1, 2,
- formulae for repeatability and reproducibility:

$$\begin{aligned} \hat{r} &= 2\sqrt{2}\sqrt{\hat{\sigma}^2} = 2.83\sqrt{0.138} = 1.05, \\ \hat{R} &= 2\sqrt{2}\sqrt{\hat{\sigma}_A^2 + \hat{\sigma}^2} = 2.83\sqrt{0.852 + 0.138} = 2.82. \end{aligned}$$

Random Effects 2-Way ANOVA

Lab example (full data):

- statistical model: ($i \sim$ lab, $j \sim$ dilution, $k \sim$ rep.):

$$y_{ijk} = \mu + A_i + \beta_j + AB_{ij} + \varepsilon_{ijk}$$

  where $A_i \sim \mathrm{N}(0, \sigma_A^2)$, $AB_{ij} \sim \mathrm{N}(0, \sigma_{AB}^2)$, $\varepsilon_{ijk} \sim \mathrm{N}(0, \sigma^2)$,

- A_i = overall (across dilutions) random effect of lab i,
- AB_{ij} = dilution-specific random effect of lab i (at j),
- ε_{ijk} = within-laboratory (and dilution) error,
- $\mathrm{Var}(y_{ijk}) = \sigma_A^2 + \sigma_{AB}^2 + \sigma^2$.

Source	DF	SS	MS	EMS	F	P
Labs	4	47.70	11.93	$\sigma^2+2\sigma_{AB}^2+6\sigma_A^2$	MSA/MSAB = 8.59	0.005
Dilu.	2	271.70	135.8	$\sigma^2+2\sigma_{AB}^2+\sigma_\beta^2$	MSB/MSAB = 97.8	< 0.001
L $*$ D	8	11.11	1.389	$\sigma^2 + 2\sigma_{AB}^2$	MSAB/MSE = 8.61	< 0.001
Error	15	2.42	0.161	σ^2		
Total	29	332.93				

$\sigma_\beta^2 = \text{constant} \times \sum_j \beta_j^2$

- all effects clearly significant $\Rightarrow$ all variance components of importance (+ dilution differences, of course!),
- estimates:

$$\begin{aligned}
\hat{\sigma}^2 &= \mathrm{MS}_E = 0.161, \\
\hat{\sigma}_{AB}^2 &= (\mathrm{MSAB} - \mathrm{MS}_E)/2 = 0.614, \\
\hat{\sigma}_A^2 &= (\mathrm{MS}_A - \mathrm{MSAB})/6 = 1.756, \\
\hat{r} &= 2\sqrt{2} \times \sqrt{\hat{\sigma}^2} = 2.83\sqrt{0.161} = 1.13, \\
\hat{R} &= 2\sqrt{2} \times \sqrt{\hat{\sigma}_A^2 + \hat{\sigma}_{AB}^2 + \hat{\sigma}^2} = 2.83\sqrt{1.756 + 0.614 + 0.161} = 4.50.
\end{aligned}$$

Overview: Analysis of Random Effects Models

- 2 types of statistical analysis:
  - ANOVA-based – "linear model methods + exceptions": *same* ANOVA table except for F-tests, explicit formulae/rules to deal with exceptions,
  - "likelihood"-based – general method: no explicit formulae, requires good software,
- 2 data situations:
  - balanced data – simple case, both methods give same results,[7]
  - unbalanced data – complex analysis, only likelihood-based methods generally work well,
- different types of software/procedures:

  a) ANOVA-based analysis, requires knowledge of specific formulae (essentially, a manual analysis): Minitab, Stata (`anova` command), SAS (`proc ANOVA`),

  b) ANOVA-based analysis but automatic analysis given the design: Minitab (`General Linear Model`) and SAS (`proc glm`), except for problems with standard errors for fixed parameter estimates,

  c) likelihood-based analysis and automatic analysis given the design: SAS (`proc mixed`), R/S-Plus (`lme` library), Stata (`mixed`), SPSS (mixed models).

[7] When using the REML version of likelihood-based analysis.

Building Random Effects Models

Multifactorial / Regression-type random effects models?
yes, sure – some guidelines and ideas:

- several random effects: indeed possible (lab. testing example) but increase complexity of model and analysis,
- random effects for predictors: possible but *difficult*,
- interaction between fixed and random effects: always random effect,
- interaction between two fixed effects: fixed[8] effect,
- nesting: (of one factor within another)
  - a (random) factor B is *nested* within A, if there is no relation between levels of B across levels of A,
  - opposite of *crossed* factors A and B,
  - model formula notation: B(A) (Stata: B|A ; R: A/B),
  - modelling: no main effect of B (as B is meaningless without A); pig breeding ex.: $y_{ijk} = \mu + \alpha_i + B_{ij} + \varepsilon_{ijk}$.

Analysis of general, balanced random effects models:

- F-tests and variance component estimation based on E(MS) formulae: use "clever" software or textbook tables,
- residuals for random effects: can be checked by looking at residuals in analysis of means for corresp. factor.

[8] (Advanced) An interaction between two fixed effects may be taken as random, to allow inference for main effects in presence of significant interaction (!!).

Stata Do-file (selection)

```
* Floral scent example
import delimited florallong.csv, clear
encode tx, gen(Tx)
anova time id per Tx
* Carton example
import delimited ch11ex2.csv, clear
keep if glue==1 & operator==1
anova strength machine
* likelihood-based analysis
mixed strength || machine:, reml
predict pred_ref, reffects
bysort machine: gen within_n=_n
qnorm pred_ref if within_n==1
* Lab example, dilution 1
import delimited add9ex1_2.csv, clear
encode lab, gen(Lab)
anova phenol Lab if dilu==1
mixed phenol || lab: if dilu==1, reml
display 2*sqrt(2)*sqrt(.138)           /* r */
display 2*sqrt(2)*sqrt(.8522501+.138)  /* R */
* Lab example, full data analysis
anova phenol Lab dilu / Lab#dilu  /* note specif. */
gen lab_dil=Lab+10*dilu
mixed phenol i.dilu || lab: || lab_dil:, reml
display 2*sqrt(2)*sqrt(.1613334)
display 2*sqrt(2)*sqrt(1.756085+.6135833+.1613334)
* Pig breeding example
import delimited add9ex4_1.csv, clear
anova wgain sire / dam|sire /* note specif. */
```