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PRACTICAL INFORMATION

Today's lecture:

- cross-over designs: based on lecture notes and 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 notes¹ on linear mixed models (random effects models), part of course curriculum.

Other news:

- home assignments: no. 3 returned today; no. 4 due March 10,
- project outline due March 14,
- exam date (April 21?).

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)
⇒ 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 ⇒ 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 learning² — 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 $\sim N(0, \sigma^2)$,
- * 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:

carry-over model	Sequence				Sequence			
	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
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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 \Rightarrow 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 \sim 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 type⁵ 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
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Bioequivalence trial (GO Example 13.10) with 12 subjects in 4 Latin squares.

Milk production example ~ cross-over trial (GO Example 13.12):

- milk yield of cows during three periods with different diets,
 - y_{ijkl} = yield for cow k in square l in period j on diet i
 - $i = A, B, C$ ~ diets (roughage, limited grain, full grain)
 - $j = 1, 2, 3$ ~ period (for each cow),
 - $k = 1, \dots, 3$ ~ cow number (within squares)
 - $l = 1, \dots, 6$ ~ Latin square number.

	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 ~ diet order AB, BC, CA; bottom ~ AC, CB, BA,
- basic statistical model (additive, no square effects),

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_{k(l)} + \varepsilon_{ijkl}, \text{ or}$$

$$y_i = \mu + \alpha_{\text{diet}(i)} + \beta_{\text{per}(i)} + \gamma_{\text{cow}(i)} + \varepsilon_i,$$

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

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 factor units (or 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							
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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>i</i>)						
<i>y_{ij}</i>	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:

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

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

- interpretations:
 - * A_i = random effect of *i*th 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*,
 - * $\text{Var}(y_{ij}) = \sigma_A^2 + \sigma^2$ (sum of two *variance components*),
- new issues for statistical analysis:
 - * estimation of σ_A^2 : $\hat{\sigma}_A^2 = (\text{MS}_A - \text{MS}_E)/n$,
(derived from the math. fact: $E(\text{MS}_A) = \sigma^2 + n\sigma_A^2$),
 - * estimation of μ : $\hat{\mu} = \bar{y}_{..}$, $\text{SE}(\hat{\mu}) = \sqrt{\text{MS}_A/(an)}$,
 - * extra model check: normality of A_i 's (using \bar{y}_i '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 ($\mu\text{g}/\text{l}$)	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 j th 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:

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

RANDOM EFFECTS 2-WAY ANOVA

Lab example (full data):

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

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

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

- 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,
- $\text{Var}(y_{ijk}) = \sigma_A^2 + \sigma_{AB}^2 + \sigma^2$.

Source	DF	SS	MS	EMS	F	P
Labs	4	47.70	11.93	$\sigma^2 + 2\sigma_{AB}^2 + 6\sigma_A^2$	$MS_A/MS_{AB} = 8.59$	0.005
Dilu.	2	271.70	135.8	$\sigma^2 + 2\sigma_{AB}^2 + \sigma_\beta^2$	$MS_B/MS_{AB} = 97.8$	< 0.001
L * D	8	11.11	1.389	$\sigma^2 + 2\sigma_{AB}^2$	$MS_{AB}/MSE = 8.61$	< 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:

$$\hat{\sigma}^2 = MS_E = 0.161,$$

$$\hat{\sigma}_{AB}^2 = (MS_{AB} - MS_E)/2 = 0.614,$$

$$\hat{\sigma}_A^2 = (MS_A - MS_{AB})/6 = 1.756,$$

$$\hat{r} = 2\sqrt{2} \times \sqrt{\hat{\sigma}^2} = 2.83\sqrt{0.161} = 1.13,$$

$$\hat{R} = 2\sqrt{2} \times \sqrt{\hat{\sigma}_A^2 + \hat{\sigma}_{AB}^2 + \hat{\sigma}^2} = 2.83\sqrt{1.756 + 0.614 + 0.161} = 4.50.$$

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: fixed⁸ 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. */
```