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PRACTICAL INFORMATION

Today's lecture: continuation of **random effects models** (from Lecture 9):

- last example from lecture 9: nested design (pig breeding data, 9L–10),
- **split-plot design:**
 - * a special type of design which is modelled/analysed by random effects models,
 - * can be viewed as a hierarchical data structure, but is constructed this way on purpose,
- any follow-up from the lab session?

Textbook reading:

- GO Chapter 12 (excluding Sections 6,7,9) and Chapter 16 (Sections 1-5); skip discussions involving Hasse diagrams¹ and restricted random effects,
- supplementary notes on linear mixed models, including split-plot designs; part of course curriculum.

Other news / practical information:

- 4th home assignment returned today; assignment # 5 posted (due March 27),
- project proposals also returned today — we can discuss today or later.

¹ Hasse diagrams is a general graphical tool to compute degrees of freedom and assess the structure of the ANOVA table for random effects models; it's not really needed for simple models but very useful for complex models.

SPLIT-PLOT DESIGN

Consider a **two-factor design with blocks or replications**:

- treatments (factors) A at a levels, and B at b levels,
- ab experimental units in each of c blocks, or replicated c times (balanced design).

Randomized (block) design:

- $N = abc$ units allocated randomly to treatments ($A \times B$), possibly within blocks,
- all treatments estimated with same precision.

Split-plot design:

- **A varies between/is applied to larger units than B** (for convenience or of necessity),
- conceptual **two-step design construction**:
 - i) A is allocated randomly to large units (“whole plots”),
 - ii) units for A are split into sub-units (“split plots”²), onto which B is allocated randomly,
- A = “whole-plot factor” and B = “split-plot factor”,
- **implication**: A estimated with less precision than B and the interaction $A*B$.
- **analysis**: include random effects for A-units (whole plots) in model!

² GO uses the term “split plots”, other authors use sub plots.

SPLIT-PLOT EXAMPLE: MEMORY TRIALS

Anxiety, tension, and memory data (GO Example 16.5):

- memory errors recorded in each of 4 memory trials taken (in random order \sim cross-over design) by 12 subjects allocated to one of four groups defined by different levels of anxiety (low/high) and muscular tension (low/high),

Subject	1	2	3	4	5	6	7	8	9	10	11	12
Anxiety	1	1	1	1	1	1	2	2	2	2	2	2
Tension	1	1	1	2	2	2	1	1	1	2	2	2
Type 1	18	19	14	16	12	18	16	18	16	19	16	16
Type 2	14	12	10	12	8	10	10	8	12	16	14	12
Type 3	12	8	6	10	6	5	8	4	6	10	10	8
Type 4	6	4	2	4	2	1	4	1	2	8	9	8

notation: y_{ijkl} = # memory errors for trial of type k for subject l with anxiety i and tension j ,

- * **whole plots** = subjects (within anxiety \times tension group),
 - * **whole-plot factors:** (A) = anxiety, (B) = (tension),
 - * **split plots** = trials (for each subject \times type),
 - * **split-plot factor** (C) = trial type,
- split-plot model with replication** (instead of blocks):

$$y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + D_{ijl} + \varepsilon_{ijkl},$$
 - * $\text{Var}(\varepsilon_{ijkl}) = \sigma^2$ (variation between split plots),
 - * $\text{Var}(D_{ijl}) = \sigma_D^2$ (extra variation between whole plots),
 - * $D_{ijl} \sim$ whole-plot random effect for subject (i, j, l) .

MEMORY EXAMPLE RESULTS

ANOVA
table:

Source	DF	SS	MS	EMS	<i>F</i>	P
Anxiety	1	10.08	10.1	$\sigma^2 + 4\sigma_D^2 + \sigma_\alpha^2$	$MS_A/MS_D = 0.98$	0.35
Tension	1	8.33	8.3	$\sigma^2 + 4\sigma_D^2 + \sigma_\beta^2$	$MS_B/MS_D = 0.81$	0.40
A * Tn	1	80.08	80.1	$\sigma^2 + 4\sigma_D^2 + \sigma_{\alpha\beta}^2$	$MS_{AB}/MS_D = 7.77$	0.024
Type	3	991.50	330.5	$\sigma^2 + \sigma_\gamma^2$	$MS_C/MS_E = 152$	<.001
A * Tp	3	8.42	2.8	$\sigma^2 + \sigma_{\alpha\gamma}^2$	$MS_{AC}/MS_E = 1.29$	0.30
Tn * Tp	3	12.17	4.1	$\sigma^2 + \sigma_{\alpha\gamma}^2$	$MS_{BC}/MS_E = 1.87$	0.16
A*Tn*Tp	3	12.75	4.3	$\sigma^2 + \sigma_{\alpha\beta\gamma}^2$	$MS_{ABC}/MS_E = 1.96$	0.15
Whole plot	8	82.50	10.3	$\sigma^2 + 4\sigma_D^2$	$MS_D/MS_E = 4.74$	<.001
Split plot	24	52.17	2.2	σ^2		
Total	47	1258.0				

- clearly significant effect of type (and no significant interactions with type),
- weakly significant interaction between anxiety and tension → interaction plot,
- variance components: $\hat{\sigma}^2 = MS_E = 2.174$, $\hat{\sigma}_D^2 = (MS_D - MS_E)/4 = 2.035$.

Some simple rules for standard errors of means, and pairwise comparisons:³

- whole-plot factors (+ interaction): usual formulae, using MS_D instead of MS_E ,⁴
- split-plot factor (type): pairwise comparisons using MS_E ,
- interactions between whole-plot and with split-plot factors: within whole-plot factor pairwise comparisons by usual formulae using MS_E .³

³ All other scenarios require approximations or mixed model software.

⁴ In a balanced design, the analysis for the whole-plot factor(s) is equivalent to analysis of whole-plot means.

CORRELATIONS IN RANDOM EFFECTS MODELS (RECAP)

Fact: random effects introduce (positive) **correlations** between the observations,

- observations are **no longer independent**, or
 - (same meaning) observations are **clustered**,
- observations **at the same level of a random factor** are correlated, whereas observations at different levels of all random factors are still independent,
- rule to compute **intra-class correlations** (ICCs):
 - * compute **the total variance**, $\text{Var}(\mathbf{y})$, of an (any) observation – as the sum of all variance components,
 - * for the two observations, \mathbf{y}_1 and \mathbf{y}_2 , in question, compute the **covariance between them**, $\text{Cov}(\mathbf{y}_1, \mathbf{y}_2)$ – as the sum of all variance components for random effects which \mathbf{y}_1 and \mathbf{y}_2 **have in common**,
 - * the **correlation** (ρ) between \mathbf{y}_1 and \mathbf{y}_2 , $\text{Corr}(\mathbf{y}_1, \mathbf{y}_2)$, is the ratio of these:

$$\rho \text{ (or ICC)} = \text{Corr}(\mathbf{y}_1, \mathbf{y}_2) = \text{Cov}(\mathbf{y}_1, \mathbf{y}_2) / \text{Var}(\mathbf{y}).$$

Example: split-plot model (with σ_{AC}^2 and σ^2 as whole-plot and split-plot variances, respectively),

$$\text{Corr}(\mathbf{y}_1, \mathbf{y}_2) = \begin{cases} \sigma_{AC}^2 / (\sigma_{AC}^2 + \sigma^2), & \text{(same whole plot),} \\ 0, & \text{(different whole plots),} \end{cases}$$

MORE ABOUT NESTING AND SUBSAMPLING

Recall **crossed versus nested** factors A and B:

- A and B are **crossed**, if every level of A and B have the same meaning across the entire experiment,
- A is **nested** within B, if levels of A are specific to levels of B and do not generalize across levels of B.

Subsampling⁴ = multiple measures taken on the same experimental unit,

- can be with “identical” subsamples (e.g. duplicates) or with factor variation,
- **split-plot designs**: split (sub) plots \sim subsampling of whole plots and allocation of split-factor treatments,
- **alternatives** to assuming independence between subsamples (rarely realistic):
 - * average observations to the level of the experimental units,
 - * include random effects \sim units subsampled.⁵

Steps to build an ANOVA model (GO Section 12.3):

1. Determine the sources of variation,
2. Decide which factors cross and which nest,
3. Decide which factors are fixed and which are random,
4. Decide which interactions should be in the model.

⁴ The (negative) term “pseudo-replication” is also used, implying not “true replication”.

⁵ To keep the original observations and add suitable random effects \sim split-plot modelling.

SPLIT-PLOT EXAMPLE: WETLAND WEED

Weed biomass in wetlands (GO Example 16.7):

- percent of nonweed biomass after 8 weeks of growth of seeds in clipping-treated halves of weed-treated wetlands placed in 8 trays, onto which nitrogen treatments were applied, positioned on 2 separate tables (in greenhouse),
- a **split-split-plot design** with blocks:

- * **whole plots** = trays (8),
- * **whole-plot factor** = N levels (4),
- * **split plots** = wetlands (24, three in each tray),
- * **split-plot factor** = weed treatment (3),
- * **split-split plots** = wetland halves (48),
- * **split-split-plot factor** = clipping treatment (2),
- * **blocks** = tables (2),

Table	N	Weed = 1		Weed = 2		Weed = 3	
		C = 1	C = 2	C = 1	C = 2	C = 1	C = 2
1	1	87.2	88.8	70.4	75.7	75.0	80.6
	2	80.5	83.8	59.2	61.5	59.5	62.5
	3	76.8	80.8	47.8	49.5	48.4	52.9
	4	77.7	81.5	35.7	37.3	38.3	42.4
2	1	78.2	80.5	65.1	68.3	65.3	66.6
	2	79.8	85.2	57.6	61.4	58.5	61.6
	3	82.4	83.1	50.5	54.0	51.6	54.7
	4	75.7	78.7	39.0	43.9	41.9	45.1

C = clipping tx

- **notation:** y_{ijkl} = biomass percentage at clipping tx k , weed tx j and nitrogen tx i in block l .

WETLAND EXAMPLE RESULTS

Model:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \delta_l + (AD)_{il} + (ABD)_{ijl} + \varepsilon_{ijkl},$$

with the **whole-plot errors** $AD_{il} \sim N(0, \sigma_{AD}^2)$, the **split-plot errors** $ABD_{ijl} \sim N(0, \sigma_{ABD}^2)$, and the **split-split-plot errors** $\varepsilon_{ijkl} \sim N(0, \sigma^2)$, and all variables independent.

Analysis:

- exact model specification difficult for ANOVA-based estimation, but possible for likelihood-based estimation (both Minitab and Stata),
- **variance components** (from REML-estimation):
$$\hat{\sigma}_{AD}^2 = 14.45, \quad \hat{\sigma}_{ABD}^2 = 2.62, \quad \hat{\sigma}^2 = 1.07,$$
- **residuals**: split-split-plot level ok, split-plot and whole-plot levels from analysis of respective means,⁶
- **interaction** n*weed clearly significant \Rightarrow interaction plot: less biomass (i.e., more weeds) with increasing N, especially when weeds have been seeded,⁷
- strong **main effect** of clipping: slight increase of biomass,
- **presentation of results**: separate for n*weed interaction and clipping main effect (because additive), by graphs or (least squares) means with standard errors.

⁶ Alternatively, one may explore the predicted random effects (BLUPs, in Stata) directly.

⁷ The interaction may be explored in the split-plot model for wetland means.

