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PRACTICAL INFORMATION

Today's lecture:

- multifactorial designs:
 - * 2-way and 3-way ANOVA: balanced (yes/no) & replication (yes/no),
 - * main effects and interaction,
- some (new) general principles for analysis.

Guidelines for textbook reading:

- you are *not* required to know how to compute SS-values in ANOVA tables (only DF-values),
- you are *not* required to compute contrasts involving more than one factor; furthermore, polynomial contrasts may often be replaced by regression modelling,
- skip too technical parts¹ of Chapters 8–10, but focus on the models and the ANOVA tables.

Other news:

- first home assignment for everyone posted,
- need to decide about final exam: April 18th?
- need to think about your project (prepare to discuss in Monday's lab, or set up an appointment next week),
- should we introduce review problems in lab sessions?

¹ Technical parts: 177₈–179¹⁵; 179₉–180₅; 181₁₁–181₄; 183₁₀–184¹¹; 184₁₆–185⁴; 192₇–194₁; 205₅–208₆; Figure 8.6; Sections 9.2.3, 9.2.4 and 9.3.

MULTI-FACTORIAL DESIGNS

Several factors in the same design?

Yes! — in good designs it is possible to separate effects of different factors from each other \Rightarrow

- possible to study combined effect of several factors (the presence of interaction),
- if interaction is absent: cheaper (less experimental units) than in several one-at-a-time experiments,²
- increased scope of the experiment,

and analysing multi-factorial data by each factor separately: is generally wrong and only gives valid results if at most one factor is of importance.

Design terminology and issues:

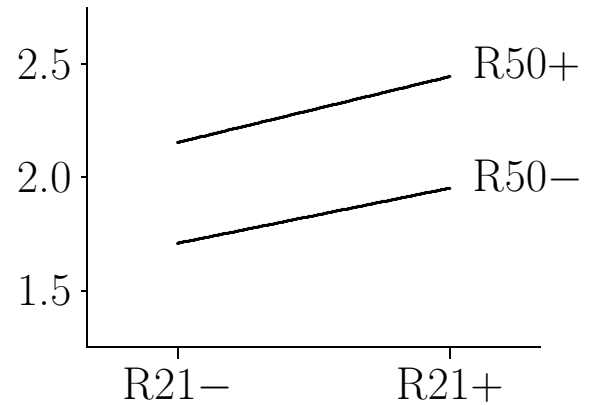
- balancedness: all (combined) groups are equally large, otherwise unbalanced,
- completeness: all (combined) groups are present (no empty cells), otherwise incomplete (*should be avoided*),
- replication: some of (combined) groups have $n > 1$, otherwise no replication (all $n = 1$),
- factorial structure can be combined with blocking structures (next lecture).

² Simple example: effects of alcohol (A: 0/1) and sleeping pills (B; 0/1). Two “one-at-a-time” studies (effect of A at fixed level of B; effect of B at fixed level of A), each with 20 subjects, give same precision as a combined study with 5 subjects per alcohol×pill combination (in the absence of interaction).

DECOMPOSING A 2-WAY TABLE OF MEANS I

Example 8.6: Nonstarter bacteria in cheddar cheese:

Total free amino acids		Strain R21		Mean
		no	yes	
Strain R50	no	1.709	1.952	1.831
	yes	2.153	2.444	2.299
Mean		1.931	2.198	2.065



Different ways to look at the data:

- (i) four separate groups,
- (ii) two R50 groups for each R21 group,
- (iii) two R21 groups for each R50 group,
- (iv) (overall level), two R50 groups, two R21 groups, association between R50 and R21 groups.

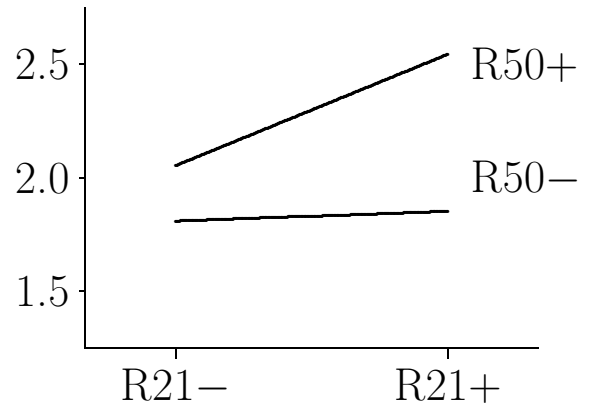
Decomposition of means corresponding to (iv):

$\bar{y}_{..}$	2.065 2.065	-0.134 0.134	$\bar{y}_{.j} - \bar{y}_{..}$
overall mean	2.065 2.065	-0.134 0.134	R21 effect
$\bar{y}_{i.} - \bar{y}_{..}$	-0.234 -0.234	0.012 -0.012	$\bar{y}_{ij} - \bar{y}_{i.}$
R50 effect	0.234 0.234	-0.012 0.012	$-\bar{y}_{.j} + \bar{y}_{..}$

DECOMPOSING A 2-WAY TABLE OF MEANS II

Modified bacteria in cheese data:

Total free amino acids		Strain R21		Mean
		no	yes	
Strain R50	no	1.809	1.852	1.831
	yes	2.053	2.544	2.299
Mean		1.931	2.198	2.065



Decomposition of means corresponding to (iv):

$\bar{y}_{..}$	2.065 2.065	-0.134 0.134	$\bar{y}_{.j} - \bar{y}_{..}$
overall mean	2.065 2.065	-0.134 0.134	R21 effect
$\bar{y}_{i.} - \bar{y}_{..}$	-0.234 -0.234	0.112 0.112	$\bar{y}_{ij} - \bar{y}_{i.}$
R50 effect	0.234 0.234	-0.112 0.112	$-\bar{y}_{.j} + \bar{y}_{..}$

Comparison of two variants of bacteria in cheese data:

- same overall level, same overall (average) effects of both R50 and R21,
- original data: almost parallel lines \Rightarrow additive effects, (same effect of one factor at all levels of other factor(s)),
- modified data: non-parallel lines \Rightarrow non-additive effects, or interaction between the factors R50 and R21.

INTERACTION AND ADDITIVITY

Interaction (synergism/antagonism, epistasy, covariation):

- the combined effect of two factors³ is not predictable from isolated effects of each of them examined separately,
- the effect of one factor depends on the level of the other factor,
- non-parallel lines in plot of means versus one factor,
- no additivity between factors⁴,
- note: interaction is dependent on scale (of outcome).

Dealing with interaction between two factors:

- decomposition of SS for combined factor $A \times B$:

$$SS_{A \times B} = SS_A + SS_B + SS_{A*B},$$

and each SS corresponds to treatment contrast(s),

- DF formula: $DF_{A*B} = DF_A \cdot DF_B$ (if $A \times B$ complete),
- in presence of an “important” interaction (significant and strong), the main effects are of no direct interest,⁵
- use interaction plot based on means or parameter estimates to understand character of interaction,
- possibly explore interesting contrasts in combined factor.

³ Interaction between three factors: the interaction between two of the factors depends on the level of the third factor.

⁴ i.e., interaction is the opposite of additivity, or additivity means no interaction.

⁵ In GO terminology (Section 8.11), the hierarchy is retained by not removing main effects involved in an interaction, so they may not need to be tested at all.

ANOVA TABLE: BACTERIA IN CHEESE DATA

Steps of ANOVA analysis:

- four treatments $\sim 2 \times 2$ factorial R50 \times R21:

$$SS_{\text{Trt}} = 3[(1.709 - 2.065)^2 + \dots + (2.444 - 2.065)^2] = 0.8723,$$

- decomposition of SS_{Trt} by orthogonal contrasts:

contrast $w(\{\mu_i\})$	w_1	w_2	w_3	w_4	\hat{w}	$SS(\hat{w})$	$F = t_w^2$
main R50	1	1	-1	-1	-0.935	0.656	7.23
main R21	1	-1	1	-1	-0.535	0.214	2.36
interaction	1	-1	-1	1	0.048	0.002	0.02

SS_{Trt}

- ANOVA table:

Source	DF	SS	MS	F	P -value
R50	1	0.656	0.656	7.23	0.028
R21	1	0.214	0.214	2.36	0.16
Interaction	1	0.002	0.002	0.02	0.89
Error	8	0.726	0.091		
Total	11	1.598			

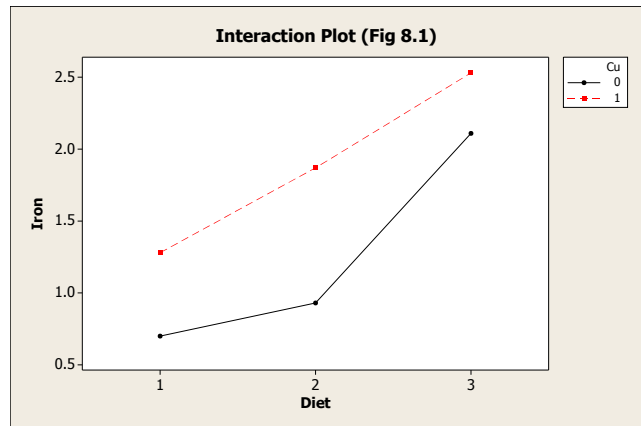
- conclusions:

- * absolutely non-significant interaction between effects of R50 and R21,
- * non-sign. main effect of R21 \Rightarrow no effect of R21 at all,
- * significant, but weak main effect of R50: adding R50 increases TFAA; illustrate by estimates, CIs or plots.

INTERACTION PLOT EXAMPLES

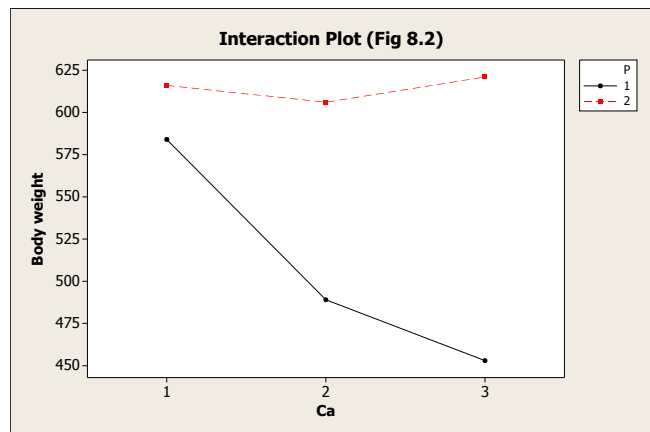
Rat data (Example 8.2):

- outcome: iron levels in liver tissue,
- factors: milk diet (3), copper deficiency (2),
- interpretation: close to additive effects.



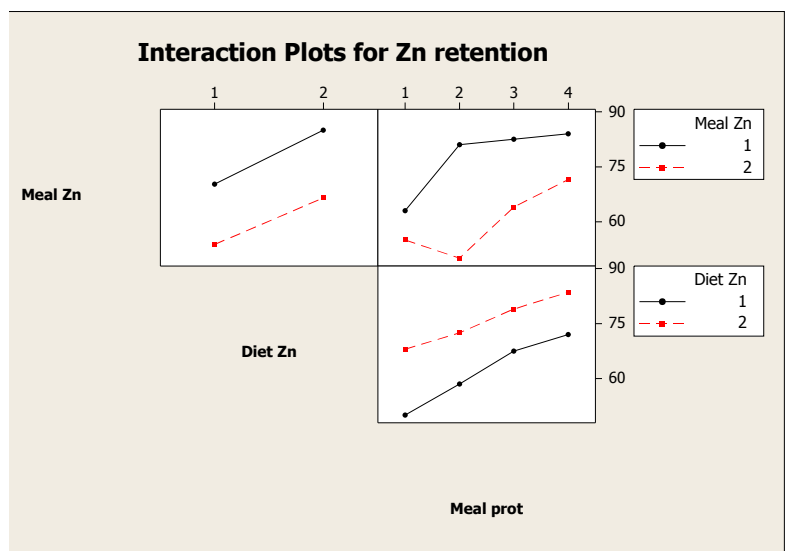
Chick data (Example 8.4):

- outcome: body weights,
- factors: Ca suppl. (3), P suppl. (2)
- interpretation: interaction: Ca effect for low P (P=1) only.



Rat data II (Example 8.5):

- outcome: Zn retention,
- factors: Diet Zn (2), final Meal Zn (2), final Meal protein (4),
- interpretation: interaction Meal Zn * Meal protein; other effects additive.



SPECIFICATION OF INTERACTION

Assume a row by columns layout of two factors, and let

$$\mu_{ij} = \mu + \alpha_i + \beta_j + \gamma_{ij},$$

where $i \sim$ rows, $j \sim$ columns, and

- μ_{ij} = mean of (i, j) th group,
- μ = overall or baseline mean (or “intercept”),
- α_i = deviation of i th row group from overall mean,
- β_j = deviation of j th column group from overall mean,
- γ_{ij} (or $(\alpha\beta)_{ij}$, GO and commonly used notation) = *interaction* = deviation of (i, j) th group from *additivity*.

Technical note: some restrictions on α 's, β 's and γ 's needed (otherwise too many parameters):

- GO & Minitab : $\sum_i \alpha_i = 0, \sum_j \beta_j = 0, \sum_i \gamma_{ij} = 0, \sum_j \gamma_{ij} = 0,$
 Stata : $\alpha_1 = 0, \beta_1 = 0, \gamma_{11}, \dots, \gamma_{1b} = 0, \gamma_{11}, \dots, \gamma_{a1} = 0,$
 SAS : $\alpha_a = 0, \beta_b = 0, \gamma_{a1}, \dots, \gamma_{ab} = 0, \gamma_{1b}, \dots, \gamma_{ab} = 0.$

Computer software formalism for factors A and B :

- Minitab (Balanced ANOVA menu), SAS:
 $A * B$ = interaction between A and B,
 $A | B = A \ B \ A * B$ (main effects and interaction).
- Stata vers. ≥ 12 : # instead of *, and ## instead of |.
- R software: : instead of *, and * instead of |.

TEXTBOOK EXAMPLES OVERVIEW

ANOVA examples of Chapters 8, 9.1-2 and 10.1-2:

- 8.6: non-starter bacteria in cheese (addition of two bacteria): 2×2 factorial with 3 replicates,
- 8.8: page faults (#) in CPU experiment (program algorithms and settings): $2 \times 3 \times 3 \times 3$ factorial with no replication (*models without replication*⁶),
- 8.10, 9.3: amylase activity in maize (analysis and growth temp., variety): $2 \times 2 \times 8$ factorial with 3 replicates, both temperatures quantitative (*polynomial contrasts*),
- 9.2: unspecified outcome and factors: 2^4 ($2 \times 2 \times 2 \times 2$) factorial with 2 replicates (*one-cell interaction*⁷),
- 9.4: seed viability (storage cond.): 3×7 factorial with 3 replicates, both factors quantitative (*polynomial contrasts*),
- 10.1-2: amylase activity (8.10) with one observation omitted (*unbalanced data*⁸),
- 10.3: unspecified outcome and factors: 2×2 factorial with highly unequal replication (*unbalanced data*⁸).

⁶ Most common method is to omit highest order interaction(s) and thus obtain estimate of residual error: conservative approach if these interactions are non-zero.

⁷ An interaction may be due to a single mean value deviating from additivity.

⁸ Unbalanced data no longer have orthogonal factorial contrasts \Rightarrow effects depend on other terms in model (as in general regression); two types of sum of squares:

- partial/adjusted SS: remove term while keep all others in;
- sequential SS: remove terms sequentially (\uparrow) so only terms above are kept in.

3-WAY ANOVA WITH REPLICATION

Amylase activity example: (GO Example 8.10)

- amylase specific activity of sprouted maize under 32 treatment conditions:

y_{ijkl} = activity for maize plant batch l of type (i, j, k)

$i = 1, \dots, 8 \sim$ analysis temperature

(10,13,15,20,25,30,35,40°C),

$j = 1, 2 \sim$ growth temperature (13,25°C),

$k = 1, 2 \sim$ variety (B73,O43),

$l = 1, 2, 3 \sim$ replicate,

- completely randomized design (if full randomization),
- full statistical model = 1-way ANOVA with 32 groups,

$$y_{ijkl} = \mu_{ijk} + \varepsilon_{ijkl},$$

where the errors $\varepsilon_{1111}, \dots, \varepsilon_{8223}$ are i.i.d. and $\sim N(0, \sigma^2)$.

- decomposition of combined factor levels into main effects and interactions (first order and second order),

$$\mu_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk},$$

* α_i = main effect of **atemp** (level i),

* $(\alpha\beta)_{ij}$ = interaction **atemp*gtemp** (levels i and j),

* $(\alpha\beta\gamma)_{ijk}$ = interaction **atemp*gtemp*var** (levels i , j and k).

- model checks based on full model \Rightarrow same approach as in 1-way ANOVA: logarithmic transform appropriate.

AMYLASE: SUMMARY OF RESULTS

ANOVA table (analysis on natural log scale):

Source	DF	Seq SS	Adj SS	Adj MS	F	P
atemp	7	3.01613	3.01613	0.43088	78.86	0.000
gtemp	1	0.00438	0.00438	0.00438	0.80	0.374
var	1	0.58957	0.58957	0.58957	107.91	0.000
atemp*gtemp	7	0.08106	0.08106	0.01158	2.12	0.054
atemp*var	7	0.02758	0.02758	0.00394	0.72	0.654
gtemp*var	1	0.08599	0.08599	0.08599	15.74	0.000
atemp*gtemp*var	7	0.04764	0.04764	0.00681	1.25	0.292
Error	64	0.34967	0.34967	0.00546		
Total	95	4.20202				

- high $R^2 = 1 - 0.3497/4.2020 = 91.7\%$,
- non-significant terms: `atemp*gtemp*var` and `atemp*var` (but not `gtemp` because involved in a significant interactions), and `atemp*gtemp` is close to significant,
- final model: `atemp*gtemp gtemp*var`, but no need to refit model (pool variance terms) because DF_E is large,
- additive effects of `atemp` and `var` for given `gtemp`,
- interaction plots: strong, parabolic-type effect of `atemp` (no obvious interpretation of `gtemp` interaction), and different effects of `gtemp` for the varieties,
- presentation of `gtemp*var` results by means with SE:

var=B73		var=O43		SE
gtemp=13	gtemp=25	gtemp=13	gtemp=25	
5.85	5.92	5.75	5.70	0.015
- `atemp` effects: multiple comp. or polynomial modelling.

AMYLASE: FURTHER ANALYSES

Polynomial modelling (GO Example 9.3):

- `atemp` contrasts not so attractive due to non-equidistant temperatures,
- for simplicity, refit with (clearly) non-significant `atemp` terms omitted before polynomial modelling,
- quadratic model in `atemp` has lack-of-fit test:

$$F = [(0.52148 - 0.42489)/(88 - 78)]/0.00545 = 1.71 \\ \sim F(10, 78) \text{ under } H_0, P = 0.094,$$

— no formal evidence against quadratic model, but higher order terms may improve fit,

- cubic model in `atemp` has lack-of-fit test:

$$F = [(0.45037 - 0.42489)/(86 - 78)]/0.00545 = 0.58 \\ \sim F(8, 78) \text{ under } H_0, P > 0.5,$$

— cubic polynomial model seems appropriate.

- interpretation of fitted model: plots of predicted curves.

Illustration: effect of unbalancedness (GO Examples 10.1-2): dropping one observation (first in dataset):

- sequential and partial/adjusted (SAS type I and III) sum of squares don't coincide: SS values for `gtemp` range within 0.00140–0.00330 across different models,
- model building must be sequential (as in regression),
- simple means are no longer best estimates (next slide).

TOOLS FOR FACTOR EFFECTS

Least squares means = tool for interpret. factor effects:

- in balanced or other “nice” designs: just simple means,
- generally: factor level estimates, while keeping all other predictors *at their average*:
 - * continuous predictors at their sample mean,
 - * categorical predictors by averaging across all levels,
- interpretation: estimated level for “average subject” wrt. all other predictors (but often not a “real” subject),
- usually better than simple means because factor levels are compared “all other things being equal”; exception is with strongly correlated predictors (difficult case),
- directly available in Minitab and SAS (in Stata via `margins` command).

The parameter estimates themselves:

- continuous predictors: always look at regression coef.,
- factors: recall the restrictions (depending on your software program); usually, `lsmeans/margins` are sufficient,
- interaction between continuous and factor: separate regression coef. for different levels of factor (beware of parameter restrictions).

MODEL SPECIFICATION

Why specify models (maybe not a silly question...)

- documentation of analysis,
- aid for development of good models, by making clear what is taken into account and what is not.

Types of model specifications — illustrated by Amylase example (`atemp`, `gtemp`, `var`):

- fully specified model, multi-index notation:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\beta\gamma)_{jk} + \varepsilon_{ijkl},$$

where $i = 1, \dots, 8$; $j, k = 1, 2$; $l = 1, 2, 3$,

- fully specified model, single-index notation:

$$y_i = \mu + \alpha_{\text{atemp}(i)} + \beta_{\text{gtemp}(i)} + \gamma_{\text{var}(i)} + (\beta\gamma)_{\text{gtemp*var}(i)} + \varepsilon_i,$$

where $i = 1, \dots, 96 \sim$ observation number,

- fully specified model, no-index notation:

$$y = \mu + \alpha_{\text{atemp}} + \beta_{\text{gtemp}} + \gamma_{\text{var}} + (\beta\gamma)_{\text{gtemp*var}} + \varepsilon,$$

- model formula (software: without +’s and error term):

$$y = \text{atemp} + \text{gtemp} + \text{var} + \text{gtemp*var} + \text{error}.$$

Regression terms / Covariates:

- in specified models: terms like $\beta_1 \cdot \text{atemp}_i + \beta_2 \cdot \text{atemp}_i^2$,
- in model formulae: special notation (options or boxes in software).

STATA DO-FILE (SELECTION)

```
import delimited ch08ta6.csv, clear /* Example 8.6 */
anova tfaa r50 r21 r50#r21 /* same with r50##r21 only */
regress /* Stata parametrization; note different P-values! */
* interaction plot
margins r50#r21
marginsplot, noci /* CIs often look messy */
* contrasts for SS decomposition computed manually
generate tx=10*r50+r21 /* combined tx variable */
anova tfaa tx
lincom 0.tx+1.tx-10.tx-11.tx /* r50 */
lincom 0.tx+10.tx-1.tx-11.tx /* r21 */
lincom 0.tx-1.tx-10.tx+11.tx /* r50*r21 */
import delimited ch08ta7.csv, clear /* Example 8.8 */
generate lnfault=ln(fault)
anova lnfault alg##seq##size##alloc /* DFE=0 */
anova lnfault alg##seq##size alg##seq##alloc alg##size##alloc
seq##size##alloc
import delimited ch10ta1.csv, clear /* Example 10.3 */
anova y a##b /* partial SS */
anova y a##b, sequential /* SS for b without interaction */
anova y b##a, sequential /* SS for a without interaction */
import delimited ch08ta9.csv, clear /* Example 8.10 */
egen tx=group(at gt v)
xi: boxcox amylase i.tx
generate lnaml=ln(amylase)
anova lnaml atemp##gtemp##v
anova lnaml atemp##gtemp gtemp##v
* atemp modelled as continuous
anova lnaml c.atemp##gtemp gtemp##v
anova lnaml c.atemp##c.atemp##gtemp gtemp##v
anova lnaml c.atemp##c.atemp##c.atemp##gtemp gtemp##v
* Example 10.1: analysis with missing value
anova lnaml atemp##gtemp gtemp##v if _n>1
margins gtemp#v, asbalanced /* least squares means */
margins gtemp#v /* all estimates different */
```