Index of Lecture 6: Multifactorial design and analysis

Practical Information

Today's lecture: expanded review of ANOVA (and regression) with some new topics: contrasts, multiple comparisons and multifactorial designs:

- two-way and three-way ANOVAs: balanced (yes/no) & replication (yes/no).

Notes on for textbook reading (i.e., the GO text):

- Chapter 3 on one-way ANOVA: mostly well-known,[1]
- Chapter 4 on contrasts: short chapter, skip Section 4.4,[2]
- Chapter 5 on multiple comparisons: more detailed than our ambition level, don't focus on mathematical details and read cursorily from Section 5.4.2 onwards,[2]
- Chapters 8–10 on multifactorial analysis: skip too technical parts[3] while focusing on the models and the ANOVA tables,
  - we are not computing SS-values in ANOVA tables manually (only DF-values),
  - you are not expected to compute contrasts involving more than one factor; furthermore, polynomial contrasts can be replaced by regression modelling.

Note: now switching completely to VHM 802 course schedule and organization.

[1] Skip Sections 3.9 and 3.11, and discussion of P(p) on p. 49.

[2] Yossa & Vardegem (2015): Misuse of multiple comparison tests and underuse of contrast procedures in aquaculture publications, *Aquaculture* 437, 344–350, may also be of interest.

[3] Technical parts: 177_8-179^{15}; 179_9-180_5; $181_{11}-181_4$; $183_{10}-184^{11}$; $184_{16}-185^4$; 192_7-194_1; 205_5-208_6; Figure 8.6; Sections 9.2.3, 9.2.4 and 9.3.

One-way ANOVA Model and Parametrization

Rat data example (GO Exercise 3.1, p. 60):

- rat liver weights in percent of body weight following four diets (labelled 1-4) randomly allocated to rats,

$$y_{ij} = \text{rat liver weight for } j\text{th rat in diet group } i,$$
$$i = 1, \ldots, g \ (g = 4); \ j = 1, \ldots, n_i \ (n_1 = 7, n_2 = n_4 = 8, n_3 = 6),$$

- purpose: assess impact of diets on liver weight.

Statistical model:

$$y_{ij} = \mu_i + \varepsilon_{ij}, \ i = 1, \ldots, g; \ j = 1, \ldots, n_i,$$

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

Model parameters (referring to underlying population):

- group means $\mu_1, \ldots, \mu_4$, and common within-group/error standard deviation σ.

Alternative formulations of same model:

$$y_i = \mu_{\text{diet}(i)} + \varepsilon_i, \quad i = 1, \ldots, 29 \ (\sim \text{observation number}),$$
$$y_i = \beta_0 + \beta_1 1_{\text{diet2}(i)} + \beta_2 1_{\text{diet3}(i)} + \beta_3 1_{\text{diet4}(i)} + \varepsilon_i,$$
$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij} \quad (\text{or, } y_i = \mu + \alpha_{\text{diet}(i)} + \varepsilon_i), \quad \text{with restrictions on } \alpha\text{'s.}^4$$

[4] Restrictions on (α_i): either $\alpha_1 = 0$ (Stata; Minitab `Regression` (default); R), $\alpha_4 = 0$ (SAS), or $\alpha_1 + \ldots + \alpha_4 = 0$ (Minitab `General Linear Model` (default)).

ANOVA versus Regression

= two different frameworks for analyzing the same model and presenting the results.[5]

Advantages of ANOVA framework:

- no reliance on an, often artificial, reference category,[6]
- extra tools for exploring multiple samples and/or multiple factors, in particular for balanced data.

Advantages of regression framework:

- easier to include continuous predictors (the equivalent of "analysis of covariance" (ANCOVA), which no longer plays any prominent role in ANOVA methods),
- full range of model checking and diagnostic tools (although some of these are of questionable value for categorical predictors; e.g. VIF and leverage).

Minitab vs. Stata: (see 6L−21 for notes on R and SAS)

- more complete regression and ANOVA facilities in Stata,
- more easily accessible facilities in Minitab.

[5] From Oehlert (p. 44): "Strictly speaking, ANOVA is an arithmetic procedure for partitioning the variability in a data set [...], however [...] we sometimes speak of testing via ANOVA although the test is not really part of the ANOVA." Other authors (e.g., Christensen 1996, p. 132) use ANOVA "as a name for the entire package of techniques used to compare more than two samples".

[6] A common mistake within the regression framework is to explore only comparisons with the reference category, cf. 4bL−10.

How to Proceed after the ANOVA Test?

We rejected overall $H_0: \mu_1 = \ldots = \mu_g$, but what relations exist between μ_i's (which differ)?

- estimation of parameters: $\hat{\mu}_i = \bar{y}_{i\cdot}$ and of derived parameters such as contrasts:

$$w(\{\mu_i\}) = \sum_{i=1}^{g} w_i \mu_i = \sum_{i=1}^{g} w_i \alpha_i, \quad \text{with } \sum_i w_i = 0,$$

  — examples (for $g = 3$, i.e. 3 groups):

  * $w(\{\mu_i\}) = \mu_1 - \mu_2$ (i.e., $w_1 = 1$, $w_2 = -1$, $w_3 = 0$),
  * $w(\{\mu_i\}) = \frac{1}{2}(\mu_2 + \mu_3) - \mu_1$ (i.e., $w = (-1, \frac{1}{2}, \frac{1}{2})$),

- confidence intervals/tests for interesting parameters, e.g., using $t^* = t(1 - \frac{\alpha}{2}, \mathrm{DF}_E)$:

$$\mu_i \ : \ \bar{y}_{i\cdot} \pm t^* \sqrt{\mathrm{MS}_E} \sqrt{(1/n_i)},$$
$$\mu_i - \mu_{i'} \ : \ \bar{y}_{i\cdot} - \bar{y}_{i'\cdot} \pm t^* \sqrt{\mathrm{MS}_E} \sqrt{(1/n_i) + (1/n_{i'})},$$ [7]

- diagram, e.g. $\hat{\mu}_i$'s with error bars (interval or margins plot),
- problems with choice of contrasts/pairwise comparisons:
  * many hypotheses; if each test has error of 5%, then total error is $\gg 5\%$,
  * above methods apply only to preplanned hypotheses, not to hypotheses suggested by the data,

  — therefore always an advantage to have other hypotheses (in addition to the overall H_0) defined prior to analysis.

[7] Note: the margin of error equals the LSD (least significant difference) for unadjusted comparisons between groups.

Multiple Comparisons: Overview

Some terminology and basic facts:

- type I (error) probability: probability of rejecting H_0, when H_0 is actually true, [7]
- per comparison or individual error rate: type I probability for each test,
- simultaneous or experimentwise or familywise error rate: type I probability for all tests, i.e., for rejection of any test in a set ("family") of tests carried out; larger than individual error rate(s),
- strong familywise error rate: probability of rejecting any true null hypotheses (but no impact of false null hypotheses/true rejections),
- multiple comparison procedures reduce the type I prob. and increase type II prob. — a trade-off,
- doing (very) many pairwise *t*-tests (LSD or Fisher method) is (very) liberal, i.e. likely to have false significances.

(Relatively) simple methods (in this course):

- Bonferroni & Holm corrections for preplanned or all comparisons,
- Scheffé's method for contrasts suggested by the data.

Other methods exist (in abundance), and can be used to control specific error rates (GO Display 5.2):

- many require balanced data (Tukey, Duncan) for exact inference,
- some are for special cases (e.g., Dunnett for comparison with control),
- some assume independent tests (e.g., Benjamini & Hochberg's false discovery rate (i.e., proportion of false rejections out of all rejections) method).

[7] Conversely, the type II (error) probability is for not rejecting H_0, when it is false.

Bonferroni Method

Idea: if we make K comparisons (tests), we can achieve the simultaneous type I probability for all tests to be $\leq \epsilon$, by taking the type I probability for each test equal to ϵ/K,[8]

- either by changing the significance level (to ϵ/K), or by multiplying P-values for individual tests by K (while keeping the significance level unchanged),
- K may be the number of preplanned comparisons, or for unplanned comparisons[9]

$$K = \text{total number of comparisons} = \binom{g}{2} = g\,(g-1)/2.$$

Notes for Bonferroni method:

- gives also simultaneous confidence intervals,[10]
- is conservative (i.e., may lead to too few hypotheses rejected) for controlling the strong familywise error rate for unplanned comparisons,
- is available for ANOVA in Minitab only via `General Linear Model` followed by `Comparisons`; in Stata, available by `pwcompare` and `test` commands,
- is flexible: applies to a wide range of settings/models.

[8] The (mathematical) justification for the Bonferroni method was explained in VHM 801 (Lecture 9).
[9] Unplanned comparisons include comparisons suggested by the data, e.g. involving the lowest/highest groups.
[10] The probability that all CIs simultaneously cover their true value is $\geq 1-\epsilon$.

Holm Method

Steps of this sequential (also called step-down) procedure:

1) sort the K unadjusted P-values as:

$$P_{(1)} \leq P_{(2)} \leq \ldots \leq P_{(K)},$$

2) for the test corresponding to the ith ordered P-value, compute the adjusted P-value: $P^H_{(i)} = P_{(i)} \times (K - i + 1)$, for $i = 1, \ldots, K$,

3) rules for significance:

(i) if $P^H_{(i)} > \epsilon \Rightarrow$ non-significant (at ϵ),

(ii) if $P^H_{(i)} \leq \epsilon$ and also all $P^H_{(j)} \leq \epsilon$ for all $j = 1, \ldots, i$, $\Rightarrow$ significant (at ϵ).

Notes for Holm method:

- controls the strong familywise error rate, and is less conservative for this than the Bonferroni method,
- does not provide simultaneous confidence intervals,
- is not available in Minitab, but can be carried out manually (by the recipe above),
- adjusted P-values (i.e., the $P^H_{(i)}$ above) are available in Stata (`test` command), but the sequential rule (ii) must be checked manually,
- is also flexible: applies to the same wide range of settings/models.

Multiple Comparisons: Rat Data Example

- group means: $\hat{\mu}_1 = 3.75,\ \hat{\mu}_2 = 3.58,\ \hat{\mu}_3 = 3.60,\ \hat{\mu}_4 = 3.92,$
- assume no preplanned hypotheses or treatment (diet) structure of interest,
- a total of $K = 4 \cdot (4-1)/2 = 6$ pairwise comparisons, shown in the table:

P-value		Multiple comparison method		
pair	order	unadjusted	Bonferroni	Holm
2 vs 4	1	.0025	.015	.015
3 vs 4	2	.0068	.041	.034
1 vs 4	3	.106	.633	.422
1 vs 2	4	.128	.768	.384
1 vs 3	5	.205	1	.409
2 vs 3	6	.869	1	.869

* same conclusions by all methods: only 4 vs 2,3 significant
* Holm $P <$ Bonferroni P (except for first P)

Significance letter coding (groups with same letter not significantly different; available in Minitab `General Linear Model` and Stata `pwcompare`):

- order group means from highest to lowest,
- designate letter a to highest group + all groups not significantly different from it,
- designate letter b to next group in the same way (but drop if same pattern as for a),
- continue through all groups,
- Rat data coding: $4^a\ 1^{ab}\ 3^b\ 2^b$.

More about Contrasts

- constructed to reflect specific (ideally, pre-defined) hypotheses,
- inference by 4-step approach from formulas for estimates and SEs, for $w = w(\{\mu_i\})$:

$$\widehat{w} = \sum_i w_i \bar{y}_{i\cdot} \quad \text{and} \quad \mathrm{SE}(\widehat{w}) = \sqrt{\mathrm{MS}_E}\sqrt{\sum_i w_i^2/n_i}\ .$$

- variation explained by contrast (out of $\mathrm{SS}_{\mathrm{Trt}}$ for the grouping/model):

  $\mathrm{SS}(w) = \widehat{w}^2/(\sum_i w_i^2/n_i) = \mathrm{MS}_E \times t_w^2$, where t_w = the t-statistic for testing $w=0$,

- orthogonal contrasts:
  - idea: contrasts that explain different parts of the variation, to allow independent interpretation,[11]
  - definition: $w = \sum_i w_i \mu_i$ and $w^* = \sum_i w_i^* \mu_i$ are orthogonal if: $\sum_i w_i w_i^*/n_i = 0$,
  - fact: there exist at most $(g-1)$ pairwise orthogonal contrasts among g groups; these are not unique,
  - example: (3 groups, equal n_i's)

$$w = \mu_1 - \tfrac{1}{2}(\mu_2+\mu_3), \quad \text{and} \quad w^* = \mu_2 - \mu_3,$$

  - main result: for orthogonal contrasts $w^{(1)}, \ldots, w^{(g-1)}$, it holds that

$$\mathrm{SS}_{\mathrm{Trt}} = \mathrm{SS}(w^{(1)}) + \mathrm{SS}(w^{(2)}) + \ldots + \mathrm{SS}(w^{(g-1)}),$$

  — splitting (decomposing) $\mathrm{SS}_{\mathrm{Trt}}$ into contrast parts.

[11] In practice, it is not always easy to find useful orthogonal contrasts, in particular in unbalanced designs.

Scheffé's Method

- corrects for examining non-preplanned contrasts[12], and therefore "allows" to test contrasts suggested by the data,
- not available in Minitab/Stata $\Rightarrow$ manual calculation.

Idea: use same procedure as with preplanned contrasts, but replace the reference distribution:

$$\text{not} \quad \frac{\widehat{w}-w}{\mathrm{SE}(\widehat{w})} \sim t(\mathrm{DF}_E), \quad \text{but} \quad [\frac{\widehat{w}-w}{\mathrm{SE}(\widehat{w})}]^2/(g-1) \sim F(g-1, \mathrm{DF}_E),$$ [13]

for example,

- test of H_0: $w = 0$ by $F = [\frac{\widehat{w}}{\mathrm{SE}(\widehat{w})}]^2/(g-1) \sim F(g-1, \mathrm{DF}_E)$,
- 95% CI for w: $\widehat{w} \pm \sqrt{(g-1)F(.95, g-1, \mathrm{DF}_E)}\,\mathrm{SE}(\widehat{w})$.

Properties:

- method can never give stronger result than overall F-test ($\mathrm{H}_0: \mu_1 = \ldots = \mu_g$),
- there always exists a contrast to give exactly same result as the overall F-test (but it is usually not interesting),
- method is conservative.

[12] Method should not be used for pairwise comparisons, because in this situation it will be very conservative.
[13] Based on the mathematical relation: $[(\widehat{w}-w)/\mathrm{SE}(\widehat{w})]^2/(g-1) \leq \mathrm{MS}_{\mathrm{TrT}}/\mathrm{MS}_E$.

Contrasts: Rat Data Example

- group means: $\hat{\mu}_1 = 3.75,\ \hat{\mu}_2 = 3.58,\ \hat{\mu}_3 = 3.60,\ \hat{\mu}_4 = 3.92$,
- choice of contrasts guided by group means (in absence of biological hypotheses),
- table of estimates and statistics:

Contrast	Weights				Estim.	Stand. err.	Variation	Tests	
definition	w_1	w_2	w_3	w_4	$\hat{w}$	$\mathrm{SE}(\hat{w})$	$\mathrm{SS}(\hat{w})$ (%)	Wald t	Scheffé F
$(\mu_1+\mu_2+\mu_3)/3-\mu_4$	1/3	1/3	1/3	−1	−0.281	0.085	0.456 (78.9%)	−3.32	3.67
$(\mu_2+\mu_3)/2-\mu_1$	−1	1/2	1/2	0	−0.157	0.094	0.114 (19.6%)	−1.66	0.92
$\mu_2-\mu_3$	0	1	−1	0	−0.018	0.110	0.001 (0.2%)	−0.17	0.01

- t-tests are assessed in $t(\mathrm{DF}_E) = t(25)$ with $t(0.975, 25) = 2.06$;
  F-tests are assessed in $F(g-1, \mathrm{DF}_E) = F(3, 25)$ with $F(0.95, 3, 25) = 2.99$.

Interpretations:

- contrast between last and first three groups $\sim$ 80% of variation, and is significant both with Wald test ($\sim$ pre-planned) and Scheffé test,
- other contrasts are far from significant (in particular with F-test),
- due to the unequal group sizes, the contrasts are not orthogonal (seen for example by their proportions out of $\mathrm{SS}_{\mathrm{Trt}}$ not adding add up to 100%).

Beyond One-way ANOVA

Methods reviewed for one-way ANOVA are generalisable to varying extent:

- in multiple regression models, each categorical predictor can/should be assessed separately unless part of interactions (some methods also important for interaction terms $\Rightarrow$ later lectures),
- construction and assessment of contrasts work for "all" regression models, except that proportion of variation explained is limited to linear models where the factor in question is "unaffected by" (orthogonal to) other effects,
- multiple comparisons are relevant in "all" regression models, but not all methods apply:
  - Bonferroni and Holm methods generally applicable,
  - many methods limited to balanced ANOVAs, and only few methods extend to GLMs,
  - Scheffé's method can be applied for Wald-type z-statistics by comparing z^2 to a $\chi^2(g-1)$ distribution, where g = number of groups,
  - principles/ideas behind adjustment (e.g. the distinction between different error rates) for multiple comparisons are general.

Multi-Factorial Designs (Recap)

Several factors in the same design? — Yes!, in good designs it is possible to separate effects of different factors from each other ⇒

- 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,[14]
- increased scope of the experiment/study,

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, or at least analyzed with care),
- replication: some of (combined) groups have $n > 1$, otherwise no replication (all $n = 1$),
- factorial structure can be combined with blocking structures (next lecture).

[14] 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 (in the absence of interaction) same precision as a combined study with 5 subjects per alcohol×pill combination.

Interaction and Additivity (Recap)

Interaction (synergism/antagonism, covariation):

- the combined effect of two factors[15] 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 interaction plot (i.e., plot of combined means versus one factor), where parallel lines $\sim$ additive effects (and hence no interaction).
- note: interaction is dependent on scale (of outcome), so affected by transformation.

Dealing with interaction between two factors:

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

$$\mathrm{SS}_{A\times B} = \mathrm{SS}_A + \mathrm{SS}_B + \mathrm{SS}_{A*B},$$

  and each SS corresponds to treatment contrast(s), as illustrated on next page,
- DF formula: $\mathrm{DF}_{A*B} = \mathrm{DF}_A \cdot \mathrm{DF}_B$ (if $A \times B$ complete),
- in presence of an "important" interaction (significant and strong), the main effects are of no direct interest[16], but contrasts in combined factor may be of interest.

[15] Interaction between 3 factors: the interaction between two of the factors depends on the level of the third factor.
[16] 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.

Balanced ANOVA Example: Bacteria in Cheese Data

Example 8.6 (p. 178): Nonstarter bacteria in cheddar cheese, a 2×2-factorial for the bacterial strains R50 and R21:

- SS_{Trt} computed from the four treatments means $(1.709, 1.952, 2.153, 2.444)$,

$$\text{SS}_{\text{Trt}} = 3[(1.709-2.065)^2 + (1.952-2.065)^2 + (2.153-2.065)^2 + (2.444-2.065)^2] = 0.872,$$

- decomposition of SS_{Trt} by orthogonal contrasts:

Contrast $w(\{\mu_i\})$	w_1	w_2	w_3	w_4	$\widehat{w}$	$\text{SS}(\widehat{w})$	$F = t_w^2$
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:

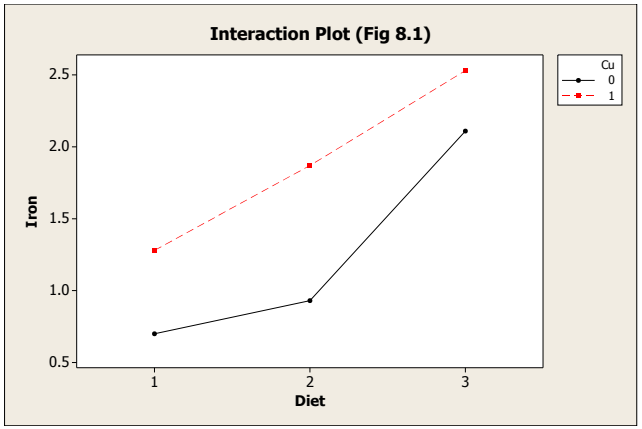
Source	DF	SS	MS	F	P-value
R50	1	0.656	0.656	7.23	0.028
R21	1	0.214	0.214	2.36	0.16
Interaction	1	0.002	0.002	0.02	0.89
Error	8	0.726	0.091		
Total	11	1.598			

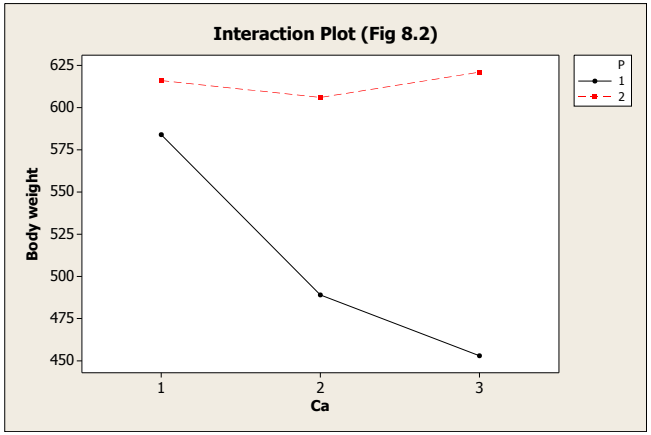
- conclusions:
  - absolutely non-significant interaction between effects of R50 and R21,
  - positive and weakly significant main effect of R50: adding R50 increases TFAA.
  - positive, but non-significant main effect of R21 $\Rightarrow$ no demonstrated R21 effect,
  - three tests are independent of each other (due to orthogonality of contrasts).

More Interaction Plot Examples

Rat data (Example 8.2):

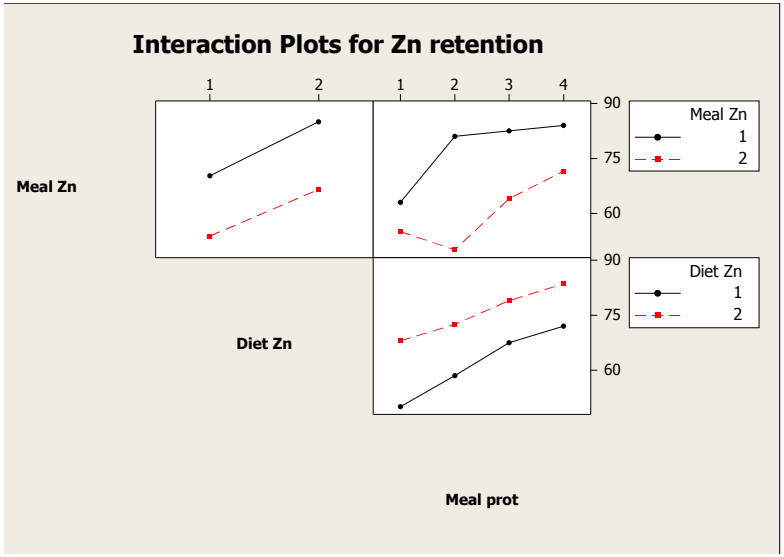
- **outcome**: iron levels in liver tissue,
- **factors**: milk diet (3), copper deficiency (2),
- **interpretation**: close to additive effects.





Chick data (Example 8.4):

- **outcome**: body weights,
- **factors**: Ca supplement (3), P supplement (2),
- **interpretation**: interaction with 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.

Textbook Examples Overview

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

- 8.6: non-starter bacteria in cheese: 2×2 factorial with 3 replicates,
- 8.8: page faults (#) in CPU experiment (program algorithms and settings): 2×3×3×3 factorial with no replication (models without replication[17]),
- 8.10, 9.3: amylase activity in maize (analysis and growth temp., variety): 2×2×8 factorial with 3 replicates, both temperatures quantitative (polynomial contrasts[18]),
- 9.2: unspecified outcome and factors: 2^4 $(2 \times 2 \times 2 \times 2)$ factorial with 2 replicates (one-cell interaction[19]),
- 9.4: seed viability (storage conditions): 3×7 factorial with 3 replicates, both factors quantitative (polynomial contrasts[18]),
- 10.1-2: amylase activity (8.10) with one observation omitted (unbalanced data[20]),
- 10.3: unspecified outcome and factors: 2×2 factorial with highly unequal replication (unbalanced data[20]).

[17] 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.

[18] For a balanced factor with quantitative (equidistant) values, contrasts ∼ polynomial terms; not in course syllabus.

[19] An interaction may be due to a single mean value deviating from additivity.

[20] Unbalanced data no longer have orthogonal factorial contrasts ⇒ 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 (↑) so only terms above are kept in.

Three-Way ANOVA with Replication

Amylase activity example (GO Example 8.10, p. 195):

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

$$\begin{aligned} y_{ijkl} &= \text{activity for maize plant batch } l \text{ of type } (i,j,k) \\ i &= 1,\ldots,8 \sim \text{analysis temperature (10,13,15,20,25,30,35,40°C)}, \\ j &= 1,2 \sim \text{growth temperature (13,25°C)}, \\ k &= 1,2 \sim \text{variety (B73,O43), and } l = 1,2,3 \sim \text{replicate}, \end{aligned}$$

- completely randomized design (if full randomization),
- full statistical model = one-way ANOVA with 32 groups: $y_{ijkl} = \mu_{ijk} + \varepsilon_{ijkl}$, where the errors $\varepsilon_{1111},\ldots,\varepsilon_{8223}$ are i.i.d. and $\sim \mathrm{N}(0,\sigma^2)$.
- 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 one-way ANOVA: demonstrating a log-transform to be appropriate.

Amylase Data: Summary of Results

ANOVA table (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, and atemp*gtemp is close to signficant,[21]
- 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		
gtemp=13	gtemp=25	gtemp=13	gtemp=25	SE
5.85	5.92	5.75	5.70	0.015

- atemp effects: multiple comparisons or polynomial modelling.

[21] Note that gtemp should not be labelled as non-significant, because it is involved in a significant interaction.

Amylase Data: Further Analyses

Polynomial modelling (GO Example 9.3):

- `atemp` contrasts not attractive due to non-equidistant temperatures,
- for simplicty, 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(s): plots of predicted curves.

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

- sequential and partial/adjusted (SAS type I and III) sum of squares do no longer 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 same as fitted means ⇒ care needed with margins.

Additional Software Notes

R analysis of one-way ANOVA and beyond:

- `oneway.test()` and `pairwise.t.test()` for one-way ANOVA with multiple comparisons,
- `lm()` and `glm()` functions for fitting linear and generalized linear models (incl. logistic regression), respectively,
- `coef()` and `vcov()` functions extract estimates and the variance-covariance matrix, respectively; further manipulation requires vector/matrix programming (e.g. using `se.contrast()` function) or pre-developed package interface,
- `multcomp` package offers wide variety of multiple comparison procedures, see documentation for use with `lm` and `glm` model fits.

SAS analysis of one-way ANOVA and beyond:

- `proc ANOVA`: one-way and multiple ANOVA,
  - limited to balanced designs,
  - includes multiple comparison methods (`means` statement),
- `proc glm`: linear models without any restrictions,
  - includes multiple comparison methods (`lsmeans` statement),
  - includes contrasts (`contrast` and `estimate` statements),
- `proc logistic` (logistic regression) and `proc genmod` (generalized linear models),
  - include contrasts (`contrast` statement), but no multiple comparisons,
- `proc multtest`: general multiple testing procedure, for linear models and import of set of unadjusted P-values.