BIOSTATISTICS VHM 802 COURSE
WINTER 2019, ATLANTIC VETERINARY COLLEGE, PEI
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Index of Lecture 12

PRACTICAL INFORMATION

Today's lecture:

- repeated measures data:
  - introduction + "warnings" + simpler methods,
  - 2 advanced methods (not part of course curriculum, but useful in practice!) — including some Stata code demonstrations.

Textbook reading – GO Section 16.6:

- brief discussion of repeated measures data with no worked examples, but good distinction between approaches,
- VER2/MER, Chapter 23: more details and (advanced) worked examples.

Supplementary material:

- notes on repeated measures: from courses taught in Denmark using SAS (lecture will demonstrate Minitab and Stata methods of analysis; SAS programming is, of course, not part of curriculum),
- lecture on repeated measures modelling (GSR Days 2013).

Schedule:

- 3rd/5th home assignment is due tomorrow,
- you should be well into your projects by now…

INTRODUCTION TO REPEATED MEASURES

Longitudinal data or Repeated measures (that is, a series of measurements) on the same observational unit (subject):

- very common; usually with repeated measures over time (e.g., observational data),
- explanatory variables may be either at time or unit level (where effect over time may be of primary interest),
- special case of *hierarchical data structure*, and of *multivariate data*,
- time points may be equidistant (designed experiments), non-equidistant but the same for all subjects, or variable between subjects (corresp. to increasing complexity),
- modelling is not easy, and stepwise approach from simple to complex models is much recommended.

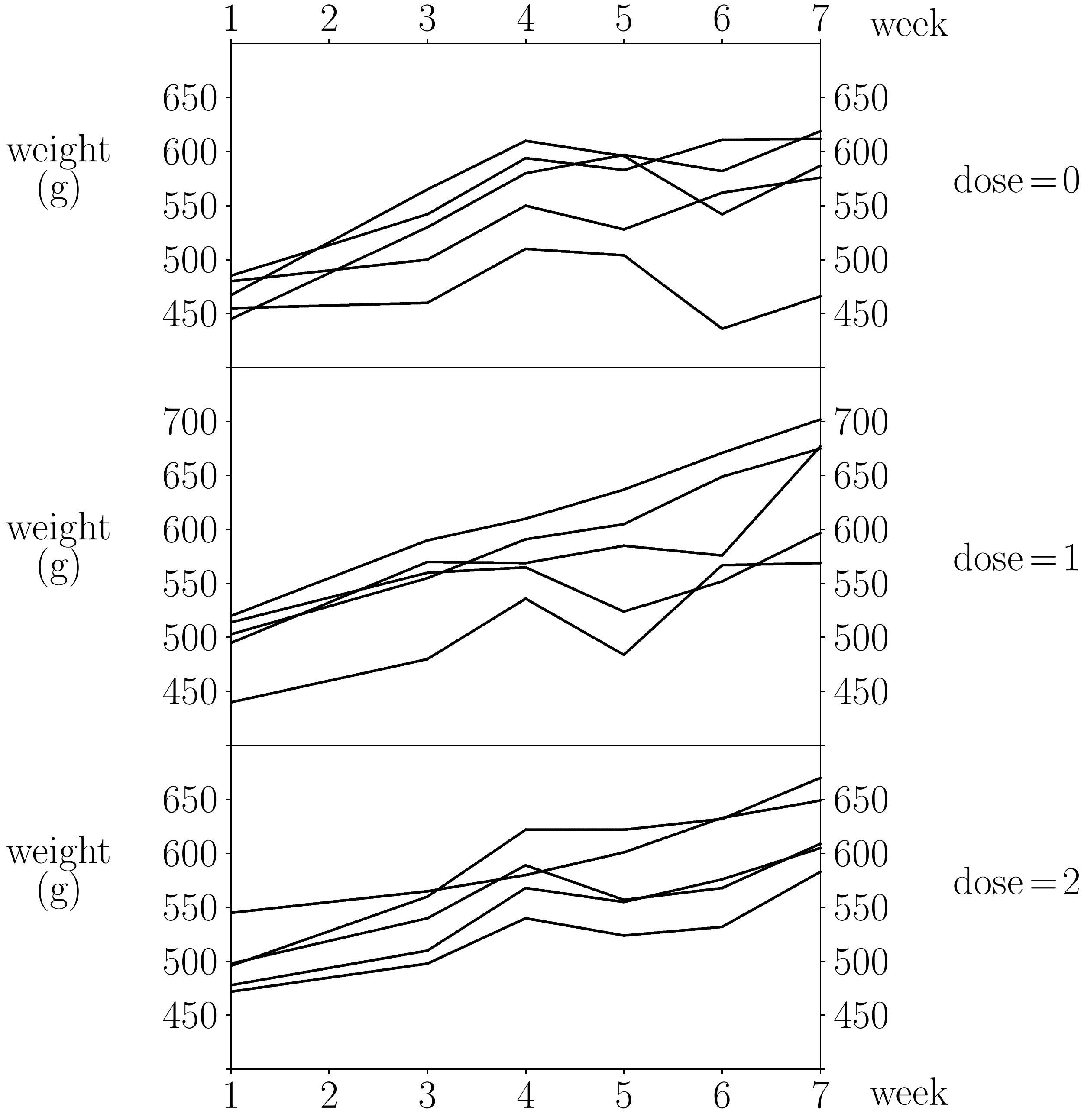
Contents of this course:

- simple univariate methods for repeated measures (separate lecture notes), except:
  - classical repeated measures ANOVA (“ϵ-correction”) available in Stata and SAS only
    ⇒ not core material in course,
- mixed models with complex correlation structures for repeated measures (available in Stata, SAS, R)
  ⇒ introduction, but not core material in course.

DATA EXAMPLE: GROWTH OF GUINEA PIGS

<u>Literature data set</u> (Crowder & Hand, 1990):

- weight of 15 guinea pigs for 7 weeks (excl. week 2),
- 3 treatment groups: 0, 1 and 2 doses of vitamine E,
- treatments applied at week 5.



STRUCTURING REPEATED MEASURES DATA

Standard notation (in the literature): double indices i and j, where $i \sim$ subject and $j \sim$ time, e.g. for Guinea pig data:

$$\begin{aligned} Y_{ij} &= \text{ weight of } i\text{th animal at time (week) } j, \\ i &= 1, \ldots, 15(m) \ \sim \ \text{animals (nested within doses)}, \\ j &= 1,2,3,4,5,6(n) \ \sim \ \text{obs. no., within animal}, \\ t_j &= \text{ week of } j\text{th obs.: } (t_1,t_2,t_3,t_4,t_5,t_6) = (1,3,4,5,6,7). \end{aligned}$$

Sometimes (e.g., VER), the meaning of indices is reversed.

Handling of incomplete series:

- the observation times (present/missing) are important: e.g., the series $(Y_{i1}, Y_{i2}, Y_{i3}, Y_{i4})$ and $(Y_{i1}, Y_{i2}, Y_{i4}, Y_{i7})$ are not the same,
- the statistical method/software should take care of this.

Data formats

Repeated measures data are frequently encountered in two different formats,

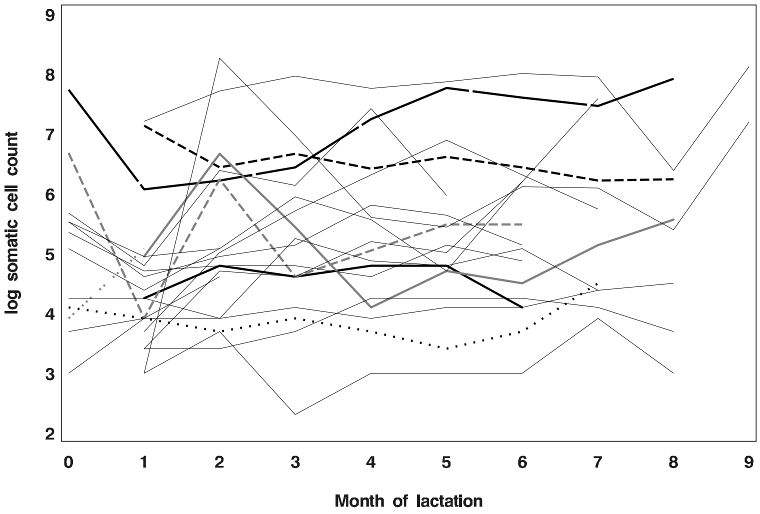
- "wide": one data row per subject and multiple columns for outcomes at different times (Guinea pig example: 15 rows, 6 columns),
- "long": one data row per measurement and columns to indicate values, subjects and times (Guinea pig example: 90 rows, 3 columns).

PLOTTING REPEATED MEASURES DATA

Two main types of plots:

- Mean plots: average values across "groups" of subjects,
  - ∗ group trends over time,
  - ∗ commonly used for presentation of results.
- Profile plots: series of values over time for subjects,
  - ∗ shows variability between subjects and consistency of mean patterns,
  - ∗ pick suitable subsample(s) if the dataset is too large to plot all profiles in one graph.

Profile plot for milk test data[1] (1 herd, 21 cows):



- highly variable patterns (both outcome and times),
- no clear effect of time is seen.

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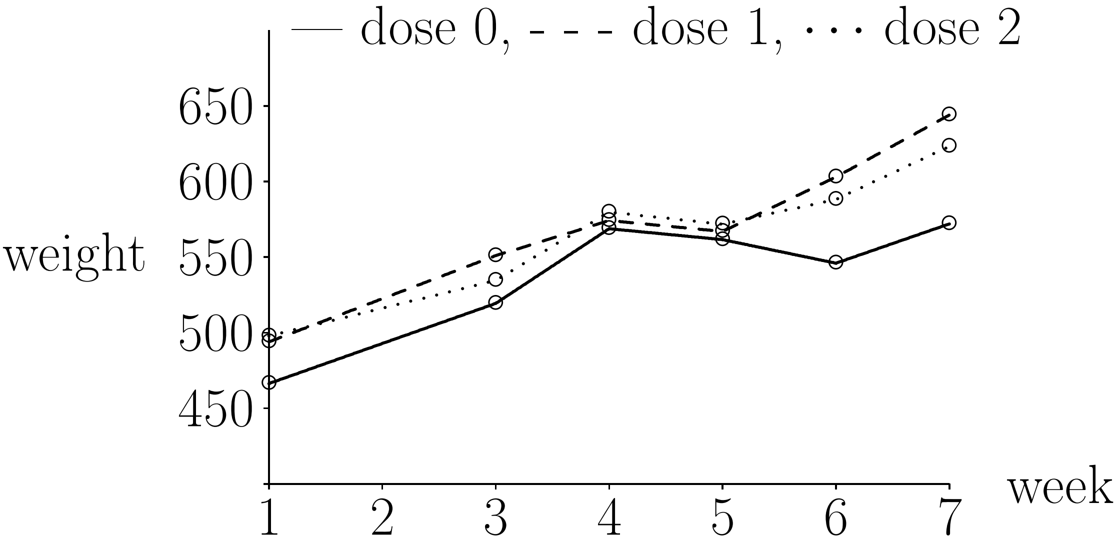
[1] Data from herd 3 in `scc_40` dataset for VER.

GUINEA PIG DATA AND MEAN PLOT

		Weeks					
Dose	Animal	1	3	4	5	6	7
0	1	455	460	510	504	436	466
	2	467	565	610	596	542	587
	3	445	530	580	597	582	619
	4	485	542	594	583	611	612
	5	480	500	550	528	562	576
1	6	514	560	565	524	552	597
	7	440	480	536	484	567	569
	8	495	570	569	585	576	677
	9	520	590	610	637	671	702
	10	503	555	591	605	649	675
2	11	496	560	622	622	632	670
	12	498	540	589	557	568	609
	13	478	510	568	555	576	605
	14	545	565	580	601	633	649
	15	472	498	540	524	532	583



UNIVARIATE METHODS FOR REPEATED MEASURES

Overview of simple univariate methods:
(primarily for balanced or close to balanced data)

0) ignore repeated measures = *disaster*; (effectively assuming all measures taken on different subjects),

i) separate analyses for different times; a *valid method* if correctly used (Bonferroni adjustment for multiple testing), but *weak method* (does not utilise full information),

ii) analysis of response feature(s) from $(Y_{i1}, \ldots, Y_{in})$, e.g.,

   - mean, slope, or curvature (higher order polynomials possible, but difficult to interpret),
   - gain = (end value − initial value),
   - area under curve (AUC);

   may be a *very effective method*, for well chosen feature,

iii) hierarchical/split-plot model with random animal effects, and

   - time = split-plot factor,
   - animal = whole plot;

   good method for short series ($n = 2, 3, 4$), but *questionable for long series*; model assumptions can be tested,

iv) repeated measures ANOVA = amendments (ε-correction) to split-plot ANOVA when it is inadequate:

   - only balanced data (incomplete series excluded, and requires same observation times for all subjects),
   - available in standard software (e.g., Stata, SAS, SPSS),
   - was a standard method for repeated measures data, but is little used today (and is only acceptable in special cases).

RESPONSE FEATURES FOR GUINEA PIG DATA

Examples of response features:

- regression coefficients:
  for each animal i, fit a regression on time (t_j's),
  $$Y_{ij} = \beta_0^{(i)} + \beta_1^{(i)} t_j + \beta_2^{(i)} t_j^2 + \ldots + \varepsilon_{ij},\ j = 1, \ldots, 6,$$
  and use $\hat{\beta}_0^{(i)}$, $\hat{\beta}_1^{(i)}$, $\hat{\beta}_2^{(i)}$, ... as features (note: only highest order coefficient useful in regression equation),
- gain after treatment onset (week 5), i.e, $d_i = Y_{i7} - Y_{i5}$.

Analysis of response features: one-way ANOVA.

	Feature (of ith animal)			
Statistic	mean $\hat{\beta}_0^{(i)}$	slope $\hat{\beta}_1^{(i)}$	curv. $\hat{\beta}_2^{(i)}$	gain d_i
avg. dose 0	539	16.0	–3.88	10.4
avg. dose 1	572	22.6	0.49	77.0
avg. dose 2	566	19.6	–0.40	51.4
SE(1-way)	17	3.6	0.85	9.3
F-test	1.06	0.83	7.45	13.0
P-value	0.38	0.46	0.008	0.001

Conclusions:

- *clearly significant difference* between group 0 and groups 1-2 for response features: gain and curvature,
- both features reflect the same pattern in the data: the growth for dose 0 animals stops after week 5.

HIERARCHICAL / SPLIT-PLOT MODEL

Repeated measures treated as hierarchical structure:

- hierarchy: measurements within subjects,
- approximate split-plot interpretation:[2]
  - ∗ animals as large units (whole plots),
  - ∗ repeated measures as sub-units (split plots),
- model: $Y_{ij} = \mu + \alpha_{\text{dose}(i)} + \beta_j + \alpha\beta_{\text{dose}(i),j} + A_i + \varepsilon_{ij}$, where α's $\sim$ dose effects, β's $\sim$ week effects, and A_i's $\sim$ animal random effects (from $\mathrm{N}(0, \sigma_A^2)$),
- assumes *same correlation between all an animal's observations*, that is, *no matter their distance in time*,
  - ∗ intuitively one would expect close observations to be more correlated than distant ones ⇒
    for long series of measures: *unreasonable assumption*,
  - ∗ for pairs ($n\!=\!2$): always ok!; for short series ($n\!=\!3, 4$): maybe not so bad,
  - ∗ fixed effects in model may add/modify correlation,
  - ∗ violation of assumption ⇒ too liberal inference for all effects involving time,
- in Guinea pig example:
  $\hat{\sigma}_A^2 = 1374$, $\hat{\sigma}^2 = 543$, $\hat{\rho} = 1374/(543+1374) = 0.72$,
- test of interaction dose×weeks: $F = 1.80$, $P = 0.08$:
  - not significant, but close (indicating "some effect"),
  - too liberal for these data; *corrected P-value* (next slide) $\approx 0.11 - 0.15$.

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[2] Contrary to the split-plot design, times are not randomized to split plots.

REPEATED MEASURES ANOVA (ϵ-CORRECTION)

= amendments to split-plot ANOVA when it is inadequate:

- test inherent assumption of equal correlations[3],
  - $*$ Mauchly's test, with reference χ^2-distribution,
- estimate seriousness of violation of the assumption by "ϵ-statistics" ($\epsilon \geq 1 \sim$ split-plot model ok),
  - $*$ $\tilde{\epsilon}$ – Huynh-Feldt statistic,
  - $*$ $\hat{\epsilon}$ – Greenhouse-Geisser statistic, (typically: $\hat{\epsilon} < \tilde{\epsilon}$)
  - $*$ ϵ^* – Box's conservative correction factor ($= 1/(n-1)$),
- possible to correct F-tests in split-plot ANOVA for violation of the assumption using the ϵ-statistics:
  - $*$ F-statistics for main effect of time and interaction time × whole-plot factor: $F(\mathrm{df}_1, \mathrm{df}_2) \rightarrow F(\epsilon\mathrm{df}_1, \epsilon\mathrm{df}_2)$,
  - $*$ F-statistic for main effect of whole-plot factor: ok!

Guinea pig example:

- Mauchly's test: statistic = 29.4, df = 14, $P = 0.0093$,
- ϵ-statistics: $\tilde{\epsilon}(\text{HF}) = 0.72$, $\hat{\epsilon}(\text{GG}) = 0.49$, $\epsilon^*(\text{Box}) = 0.20$,
- P-val (df's):

Source	split-plot	HF-corr.	GG-corr.	Box corr.
weeks	<0.001	<0.001	<0.001	<0.001
weeks×groups	0.080^{10}_{60}	0.11^{7}_{43}	0.15^{5}_{30}	0.21^{2}_{12}

- Conclusion: clear violation, but not very consequential.

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[3] (technical): the split-plot analysis is valid under a less restrictive assumption of "sphericity", and the methods really focus on deviations from sphericity.

MIXED MODELS[4] FOR REPEATED MEASURES

Idea: extend linear model to allow *correlation* among ε's within subjects (animals) in models of the form,

$$Y_{ij} = \beta_0 + \beta_1 x_{1ij} + \ldots + \beta_k x_{kij} + \varepsilon_{ij},$$

- different correlation structures possible (flexible models),
- random effects may be included/added to model above,
- possible to test nested models for the correlation structure using *likelihood-ratio test*:
  compute differences in $-2\ln L$, and compare to χ^2-distribution with df = difference in # parameters,
- recomm.: parsimonious modelling of corr. structure.

Guinea pig example:
(assuming, for simplicity, equidistant times $1-6$)

structure	# random params.	$-2\ln L$	AIC	P-value W×G
comp. symmetry	2	720.0	724.0	0.080
autoregressive (1)	2	708.6	712.6	0.16
random eff. + ar(1)	3	708.1	714.1	0.17
Toeplitz	6	698.4	710.4	0.054
unstructured	21	661.4	703.4	0.016

W×G ~ weeks×groups interaction, P ~SAS

Conclusions:

- poor fit by comp. symmetry; best fit by unstructured,
- substantial impact on P-values for fixed effect (W×G).

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[4] Also also called *covariance pattern models* when not truly “mixed”.

COVARIANCE AND CORRELATION MATRICES

For a series $Y = (Y_1, \ldots, Y_n)$ of observations on a subject[5], the <u>covariance matrix</u> $\mathrm{Cov}(Y)$ and <u>correlation matrix</u> $\mathrm{Corr}(Y)$ are the $n \times n$-matrices of all pairs of covariances[6] and correlations,

$$\mathrm{Cov}(Y) = \begin{pmatrix} \mathrm{Var}(Y_1) & & & & \\ \mathrm{Cov}(Y_1, Y_2) & \mathrm{Var}(Y_2) & & & \\ \mathrm{Cov}(Y_1, Y_3) & \mathrm{Cov}(Y_2, Y_3) & \mathrm{Var}(Y_3) & & \\ \vdots & \vdots & \vdots & \vdots & \\ \mathrm{Cov}(Y_1, Y_n) & \mathrm{Cov}(Y_2, Y_n) & \mathrm{Cov}(Y_3, Y_n) & \cdots & \mathrm{Var}(Y_n) \end{pmatrix},$$

$$\mathrm{Corr}(Y) = \begin{pmatrix} 1 & & & & \\ \mathrm{Corr}(Y_1, Y_2) & 1 & & & \\ \mathrm{Corr}(Y_1, Y_3) & \mathrm{Corr}(Y_2, Y_3) & 1 & & \\ \vdots & \vdots & \vdots & \vdots & \\ \mathrm{Corr}(Y_1, Y_n) & \mathrm{Corr}(Y_2, Y_n) & \mathrm{Corr}(Y_3, Y_n) & \cdots & 1 \end{pmatrix},$$

<u>note:</u> the matrices are symmetric, so for clarity the values above the diagonal are left blank.

<u>Simplest example</u>: independent/uncorrelated observations with the same variance σ^2 (shown for 4 observations):

$$\mathrm{Cov}(Y) = \begin{pmatrix} \sigma^2 & & & \\ 0 & \sigma^2 & & \\ 0 & 0 & \sigma^2 & \\ 0 & 0 & 0 & \sigma^2 \end{pmatrix}, \ \mathrm{Corr}(Y) = \begin{pmatrix} 1 & & & \\ 0 & 1 & & \\ 0 & 0 & 1 & \\ 0 & 0 & 0 & 1 \end{pmatrix}.$$

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[5] We suppress the subject's index i to ease the notation.

[6] Formally, $\mathrm{Cov}(Y_1, Y_2) = \mathrm{E}(Y_1 Y_2) - (\mathrm{E}Y_1)(\mathrm{E}Y_2)$, and $\mathrm{Cov}(Y_1, Y_1) = \mathrm{Var}(Y_1)$, as well as $\mathrm{Corr}(Y_1, Y_2) = \mathrm{Cov}(Y_1, Y_2)/\sqrt{\mathrm{Var}(Y_1) \cdot \mathrm{Var}(Y_2)}$.

REPEATED MEASURES CORRELATION STRUCTURES

Examples of covariance/correlation structures (4×4-matrices) for observations with homogeneous variances:

- compound symmetry/exchangeable ∼ hierarchical model,

$$\mathrm{Cov}(Y) = \begin{pmatrix} \sigma^2+\sigma_c^2 & & & \\ \sigma_c^2 & \sigma^2+\sigma_c^2 & & \\ \sigma_c^2 & \sigma_c^2 & \sigma^2+\sigma_c^2 & \\ \sigma_c^2 & \sigma_c^2 & \sigma_c^2 & \sigma^2+\sigma_c^2 \end{pmatrix}, \mathrm{Corr}(Y) = \begin{pmatrix} 1 & & & \\ \rho & 1 & & \\ \rho & \rho & 1 & \\ \rho & \rho & \rho & 1 \end{pmatrix},$$

  where $\rho = \sigma_c^2/(\sigma^2+\sigma_c^2)$ (= ICC),

- autoregressive (first order, often written `ar(1)`),

$$\mathrm{Cov}(Y) = \begin{pmatrix} \sigma^2 & & & \\ \rho\sigma^2 & \sigma^2 & & \\ \rho^2\sigma^2 & \rho\sigma^2 & \sigma^2 & \\ \rho^3\sigma^2 & \rho^2\sigma^2 & \rho\sigma^2 & \sigma^2 \end{pmatrix}, \mathrm{Corr}(Y) = \begin{pmatrix} 1 & & & \\ \rho & 1 & & \\ \rho^2 & \rho & 1 & \\ \rho^3 & \rho^2 & \rho & 1 \end{pmatrix},$$

- Toeplitz ("stationary"),

$$\mathrm{Cov}(Y) = \begin{pmatrix} \sigma^2 & & & \\ \rho_1\sigma^2 & \sigma^2 & & \\ \rho_2\sigma^2 & \rho_1\sigma^2 & \sigma^2 & \\ \rho_3\sigma^2 & \rho_2\sigma^2 & \rho_1\sigma^2 & \sigma^2 \end{pmatrix}, \mathrm{Corr}(Y) = \begin{pmatrix} 1 & & & \\ \rho_1 & 1 & & \\ \rho_2 & \rho_1 & 1 & \\ \rho_3 & \rho_2 & \rho_1 & 1 \end{pmatrix},$$

- unstructured (with inhomogeneous variance; note: $\sigma_{ij} = \sigma_i\sigma_j\rho_{ij}$),

$$\mathrm{Cov}(Y) = \begin{pmatrix} \sigma_1^2 & & & \\ \sigma_{12} & \sigma_2^2 & & \\ \sigma_{13} & \sigma_{23} & \sigma_3^2 & \\ \sigma_{14} & \sigma_{24} & \sigma_{34} & \sigma_4^2 \end{pmatrix}, \mathrm{Corr}(Y) = \begin{pmatrix} 1 & & & \\ \rho_{12} & 1 & & \\ \rho_{13} & \rho_{23} & 1 & \\ \rho_{14} & \rho_{24} & \rho_{34} & 1 \end{pmatrix}.$$

Stata Do-file

```
version 15 /* works also with versions 13-14 */
import delimited guinea_long.csv, clear
* profile plots
twoway (connected weight week), by(group  animal)
* demonstration of xtline command
xtline weight, i(animal) t(week)
xtline weight, i(animal) t(week) overlay
* profile plot for first group only
xtline weight if group==1, i(animal) t(week) overlay
* alternative use of add-on overlay command
sort animal week
overlay weight week, by(animal) c(l)
overlay weight week if group==1, by(animal) c(l) /* similar for groups 2,3 */
* mean plot
preserve /* run together with next 4 lines */
collapse (mean) weight, by(group week)
xtline weight, i(group) t(week)
overlay weight week, by(group) c(l)
restore

* disregarding repeated measures (wrong!)
anova weight group##week
* separate analyses for each week
bysort week: oneway weight group

* response features
* mean
preserve /* run together with next 3 lines */
collapse (mean) weight, by(group animal)
oneway weight group
restore
* gain
preserve /* run together with next 4 lines */
import delimited guinea_wide.csv, clear
gen gain=w7-w5
oneway gain group
restore
* slope
anova weight animal c.week#animal
regress
matrix parms_slp=e(b)'     /* creates a column of all estimates from */
svmat parms_slp            /* which the relevant ones must be extracted */
preserve /* run together with next 4 lines */
```

```
drop if _n<16 | _n>30
egen float group2 = seq(), from(1) to(3) block(5)
oneway parms_slp1 group2
restore
* curvature
anova weight animal c.week#animal c.week#c.week#animal
regress
matrix parms_crv=e(b)’     /* creates a column of all estimates from */
svmat parms_crv            /* which the relevant ones must be extracted */
preserve /* run together with next 4 lines */
drop if _n<31 | _n>45
egen float group2 = seq(), from(1) to(3) block(5)
oneway parms_crv1 group2
restore

* split-plot or hierarchical model
anova weight group / animal|group week##group
mixed weight group##week || animal:, reml
testparm group#week /* note: P-values smaller (too small) in chi^2-distrib */
mixed weight group##week || animal:, reml dfm(satterth)
* better P-values, also with dfm(kroger)˜possibly also small changes in estimates
mixed, dftable(pvalue)
testparm group#week, df(60)

* repeated measures ANOVA
anova weight group / animal|group week##group, repeated(week)

* mixed models with correlation structure, assuming equidistant times
gen weekeq=week+(week==1) /* equidistant time points 2-7*/
* ar(1) autoregressive correlations
* note: all model fits could use dfm option
mixed weight group##weekeq || animal:, res(ar 1, t(weekeq)) nocons reml
estat wcor
* random effects and ar(1) errors
mixed weight group##weekeq || animal:, res(ar 1, t(weekeq)) reml
estat wcor
* Toeplitz
mixed weight group##weekeq || animal:, res(toeplitz, t(weekeq)) nocons reml
estat wcor
* unstructured (not in lecture)
mixed weight group##weekeq || animal:, res(uns, t(weekeq)) nocons reml
estat wcor
* models with non-equidistant time points: replace weekeq by week
* example: ar(1) (not in lecture)
mixed weight group##week || animal:, res(ar 1, t(week)) nocons reml
estat wcor
```