Biostats VHM 802 Course
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Henrik Stryhn

Exercises for Session 7: Multifactorial and block designs

Exercise 7.1

Two-way analysis of variance

In an experiment with rats feeding on fat (from pigs, i.e. lard), among other things the difference in feed consumption between fresh and rancid fat was studied. The consumption during 73 days for 12 rats (6 males and 6 females) of an age between 30 and 34 days are listed in the table below, measured in *g*. (Data from Powick, W. C. (1925): Inactivation of vitamin A by rancid fat, *J. Agric. Res.* **31**, 1017-1027.)

	Fat					
Sex	fresh			rancid		
male	709	679	699	592	538	476
female	657	594	677	508	505	539

Describe the type of experiment, formulate a corresponding statistical model, and analyze the data. Draw conclusions and think about how to present the results.

Exercise 7.2

Latin square

The effect of insulin on the blood concentration of glucose was studied on rabbits. Three rabbits each received insulin doses A, B, and C (corresponding respectively to 0, 1, and 2 units) at different days. The experimental design is given below with the glucose measurements (*mg* pr. 100 *ml* blod) taken 50 minutes after injection. (Data from Young & Romans (1948): Assay of insulin with one blood sample per rabbit per day, *Biometrics* **4**, 122-131.)

	Rabbit					
Day	1		2		3	
1	A	50	C	39	B	36
2	C	37	B	51	A	53
3	B	51	A	60	C	37

Give the type of experimental design, formulate a statistical model and analyze the data. Examine whether the effect of insulin can be described as a linear function of the dose. If so, determine a 95% confidence interval for the regression coefficient.

Exercise 7.3

Multi-way ANOVA

An experiment compared three thiosemicarbazon-type preparations against virus:

I: p-amino
II: substituent p-methoxy
III: unsubstituted.

For each preparation six eggs were infected with virus solutions in three concentrations. The entire experiment was repeated once. If $x_1, \dots x_6$ denotes the survival times (hours) of the virus in the six eggs, we let

$$y = \frac{10^4}{6}\left(\frac{1}{x_1} + \frac{1}{x_2} + \frac{1}{x_3} + \frac{1}{x_4} + \frac{1}{x_5} + \frac{1}{x_6}\right).$$

The table below gives y-values for every preparation in its three concentrations at the two replications of the experiment. (Data from Hamre *et al.* (1951): Studies on the chemotherapy of vaccinia virus II: The activity of some thiosemicarbazones, *J. Immunology* **67**, 305–312.)

y	Replication 1			Replication 2		
	Concentration			Concentration		
Preparation	$10^{-4.0}$	$10^{-4.3}$	$10^{-4.6}$	$10^{-4.0}$	$10^{-4.3}$	$10^{-4.6}$
I	87	79	77	90	80	81
II	82	73	72	71	72	68
III	72	70	62	77	66	61

Describe the experimental design, formulate a statistical model, and analyze the data.

Exercise 7.4

Multi-way ANOVA

Consider time to intoxication by cyanide in *Phoxinus laevis*, a European minnow, using 5 concentrations of CN^- ions, 3 oxygen concentrations, and 3 temperatures, as follows:

temperature (°C):	5, 15, 25,
oxygen concentration (mg O_2/l):	1.5, 3.0, 9.0,
cyanide concentration (mg CN^-/l):	0.16, 0.8, 4.0, 20.0, 100.0.

The dependent variable is a transformation into logarithms of readings in minutes of survival time, coded and averaged for 10 replicate fishes. (From Sokal & Rohlf (1995): *Biometry*; data from Wuhrmann, K. & Woker, H. (1953): Über die Giftwirkungen von Ammoniak und Zyanidlösungen mit verschiedener Sauerstoffspannung und Temperatur auf Fische, *Schweiz. Z. Hydrol.* **15**, 235-260.)

		Cyanide concentration				
Temperature	Oxygen concentration	0.16	0.8	4.0	20.0	100
5	1.5	20.1	15.0	13.1	13.0	9.7
	3.0	24.6	16.4	13.8	13.6	10.2
	9.0	27.1	17.0	14.9	12.7	9.9
15	1.5	12.4	10.4	8.6	8.9	6.0
	3.0	15.8	11.1	9.9	9.1	7.4
	9.0	20.7	11.7	8.1	8.7	7.2
25	1.5	7.9	6.3	5.0	5.1	3.2
	3.0	12.9	5.4	5.1	5.2	4.6
	9.0	14.2	9.3	6.2	5.1	5.2

State the type of experimental design and suggest statistical models for the data. Note, that it may be of interest to model the data in terms of the quantitative factor levels. Analyze the data and draw conclusions.

Exercise 7.5
Planning of experiments
This exercise is based on the Checklist for Planning of Experiments from Dean & Voss: *Design and Analysis of Experiments*.

Part I
Read critically through the discussion of the Battery experiment in Section 2.5.2 of Dean & Voss. Pay particular attention to the identification of the sources of variation. Think about whether your planning of a similar experiment would have involved the same considerations and the same choices.

Part II
Go through steps (a)–(d) of the checklist for an experiment outlined as follows (by Clifford Pugh, *Applied Statistics*, 1953):

> The widespread use of detergents for domestic dish washing makes it desirable for manufacturers to carry out tests to evaluate the performance of their products. [...] Since foaming is regarded as the main criterion of performance, the measure adopted is the number of plates washed before the foam is reduced to a thin surface layer. The five main factors which may affect the number of plates washed by a given product are (i) the concentration of the detergent, (ii) the temperature of the water, (iii) the hardness of the water, (iv) the type of "soil' on the plates, and (v) the method of washing used by the operator. [...] The difficulty in standardizing the soil is overcome by using the plates from a works canteen (cafeteria) for the test and adopting a block technique in which plates from any one course forms a block. [...] One practical limitation is the number of plates available in any one block. This permits only four [...] tests to be completed (in a block).

Exercise 7.6
Unbalanced (incomplete) two-way ANOVA
The following experiment was carried out by Dr. H. Wolffhechel, KVL (Danish Veterinary and Agricultural University), in 1986. The purpose was to compare 12 sphagnum moss lots with respect to water and air content. Each of these was applied to four pots with small cucumber plants. The pots were placed in one of six watering troughs, each containing eight pots. The experimental design and the volume (water and air content), in percent, for each pot is given in the table. (From Skovgaard (1994): *Statistisk Forsøgsplanlægning*, in Danish).

Volume (percent)	Watering trough					
Sphagnum moss lot	1	2	3	4	5	6
1	37.0		44.6		42.5	47.1
2			49.0	50.5	51.0	44.8
3		34.6	42.7	41.8	37.8	
4	45.3	42.7	47.7	42.8		
5	32.1	38.5		32.0		31.6
6	34.3	33.3		34.0	22.6	
7	32.3		28.1	28.1	32.3	
8	38.9	36.5		39.7		34.8
9	33.9		31.4	32.1		23.0
10		39.7	41.8		43.5	33.8
11		41.1	38.1		31.1	37.9
12	35.9	7.5			36.2	25.5

Describe the experimental design, and explain why it is not a balanced incomplete block design. Analyse the data using Minitab/Stata; hereby, determine the error sum of squares, SSE, and degrees of freedom, DFE, for the following 4 models,

$$\begin{aligned}
&(A) \quad y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}, \\
&(B) \quad y_{ij} = \mu + \beta_j + \varepsilon_{ij}, \\
&(C) \quad y_{ij} = \mu + \alpha_i + \varepsilon_{ij}, \\
&(D) \quad y_{ij} = \mu + \varepsilon_{ij},
\end{aligned}$$

where indices i and j correspond to sphagnum moss lots and watering troughs, respectively. Test the reductions of model (A). Make sure you understand the difference between partial (adjusted) and sequential sum of squares, and state which sum of squares you would use to assess lot and trough effects.

In model (A), compare estimation of sphagnum moss lot levels by ordinary means and least squares means. Why do they differ — and which one would you use to summarise the treatment effects? Describe the relationship between the sphagnum moss lots, if necessary using statistical comparison techniques.

Exercise 7.7
ANOVA and transformation
A dataset analysed in a seminal statistical paper by Box & Cox consisted of survival times of 48 animals that were given one of 3 different poisons and prior to the poisoning had been subjected to one of 4 (unspecified) treatments. The data are shown in the table below.

Survival in hours	Treatment			
Poison	A	B	C	D
I	3.1	8.2	4.3	4.5
	4.5	11.0	4.5	7.1
	4.6	8.8	6.3	6.6
	4.3	7.2	7.6	6.2
II	3.6	5.8	4.4	5.6
	2.9	6.1	3.5	10.2
	4.0	4.9	3.1	7.1
	2.3	12.4	4.0	3.8
III	2.2	3.0	2.3	3.0
	2.1	3.7	2.5	3.6
	1.8	3.8	2.4	3.1
	2.3	2.9	2.2	3.3

Analyse the data, while paying particular attention to the points below.

1) Discuss whether it would seem appropriate to model the survival times on a transformed scale, and in that case which transformation to use. Carry out all subsequent analyses at the chosen scale.

2) Discuss whether there seems to be outlier(s) present in the data, and substantiate your discussion by statistical test(s).

3) Draw conclusions about the effects of treatments and poisons. For the quantitative comparison of poisons, assume poison III to be of one particular — and new — type, and poisons I and II to be similar and of another type, so that it is of interest to compare the two types of poison. Among the 4 treatments, no special relations exist.

4) Based on your final model, estimate with a 95% confidence interval the expected survival time of animals poisoned with poison III. Same question for the survival time of animals given treatment A and poison III.

Exercise 7.8

Several regression lines

An experiment about the accumulation of salts was conducted by measuring the concentration of rubidium and bromide ions in potato slices after the potatoes had been immersed in a solution containing these ions for several hours. The concentrations are given in the table below, in mg per 1000 g of water in the tissue. (Data from Steward, F. C. & Harrison, J. A. (1939): The absorption and accumuluation of salts by living plant cells, *Annals of Botany*, New Series **3**, 427–453.)

Duration of immersion hours	Rubidium conc. $mg/1000\ g$	Bromide conc. $mg/1000\ g$
21.7	7.2	0.7
46.0	11.4	6.4
67.0	14.2	9.9
90.2	19.1	12.8
95.5	20.0	15.8

Biochemical considerations might indicate the absorptions of bromide and rubidium ions to follow similar patterns.

1) Estimate first two separate linear regression models, one for each of the ions.

2) It is of interest to analyze the two ions in a combined statistical model. Formulate a combined model with separate regression equations for the two ions, and estimate the parameters. Which additional assumptions are made about the data compared to the analyses in 1) ?

3) Use a statistical test to assess whether the data show evidence against equal rates of absorption for the two ions. If not, estimate the parameters of a model assuming equal rates of absorption.

4) Finally, examine whether the regression equations for bromide and rubidium ions are identical. Summarize the statistical analysis and give the parameter estimates of the best model you found, with associated standard errors.

Exercise 7.9

Regression and analysis of covariance

The table below gives nave height and total height, both in feet, for medieval English cathedrals. In addition, the cathedrals can be classified according to their architectural style, either Romanesque or Gothic. Some cathedrals have both a Gothic and Romanesque part, each of different height; these cathedrals are included twice. (Data fra Weisberg (1985): *Applied Linear Regression.*)

Romanesque cathedral	height feet	length feet	*Gothic* cathedral	height feet	length feet
Durham	75	502	York	100	519
Canterbury	80	522	Bath	75	225
Gloucester	68	425	Bristol	52	300
Hereford	64	344	Chichester	62	418
Norwich	83	407	Exeter	68	409
Peterborough	80	451	Gloucester	86	425
St. Albans	70	551	Lichfield	57	370
Winchester	76	530	Lincoln	82	506
Ely	74	547	Norwich	72	407
			Ripon	88	295
			Southwark	55	273
			Wells	67	415
			St. Asaph	45	182
			Winchester	103	530
			Old St. Paul	103	611
			Salisbury	84	473

Our aim is to investigate the relation between height and length, and whether this relationship depends on architectural style. Analyse the logarithm of the length as a function of the logarithm of the height, and eliminate the cathedrals of Bath and Ripon from the analysis. (Here we take these choices for granted, but you are invited to analyse the data from scratch yourself...)

1) Formulate a statistical model where the (logarithmic) height enters as a regression variable and the architectural style as a factor. Based on this model, do the relations between height and length seem to differ for the two architectural styles?

2) Extend the model with a quadratic term — in ln(height), and repeat question 1). Compare the answers.

3) Examine the two models more closely using regression diagnostics and model checks, and possibly by analysing additional models. Do you understand better now the results obtained in 1) and 2)?