Solution to Exercise 8.6 (Version 1, 30/6/15)

from Statistical Methods in Biology: Design & Analysis of Experiments and Regression (2014) S.J. Welham, S.A. Gezan, S.J. Clark & A. Mead. Chapman & Hall/CRC Press, Boca Raton, Florida. ISBN: 978-1-4398-0878-8

Exercise 8.6 (Data: courtesy R. Curtis, Rothamsted Research/Bionemax)

An experiment assessed the effect of two lectins, Con-A and GNA, on nematode motility. Nematodes were incubated overnight with one of the two lectins or a buffer solution (PBS) as a control (factor Treatment). Nematodes were placed in the centre of Petri dishes, with four dishes allocated to each treatment completely at random. Here we analyse the total distance moved by the nematodes in each dish after 40 minutes. File NEMATODES.DAT contains the unit numbers (*DDish*), explanatory factor (Treatment) and distances moved (variate *Distance*). Analyse these data and construct contrasts to assess whether (a) addition of lectins affects nematode movement and (b) the two lectins have similar effects on movement.

Data 8.6 (NEMATODES.DAT)

Total distance moved in 40 minutes by nematodes in dishes with three types of solution:

DDish	Treatment	Distance
1	GNA	3.2
2	GNA	4.2
3	GNA	4.3
4	GNA	5.6
5	CONA	3.0
6	CONA	4.2
7	CONA	3.5
8	CONA	4.8
9	PBS	5.6
10	PBS	6.5
11	PBS	6.0
12	PBS	7.0

Solution 8.6

This is a Completely Randomized Design (CRD), with no structure in the set of experimental units (dishes). There are three experimental treatments applied, with allocation given by the Treatment factor. We can write a model for this experiment as

Response variate:	*Distance*
Structural component:	DDish
Explanatory component:	[1] + Treatment

Residual plots based on standardized residuals from this analysis are shown in Figure S8.6.1. This is

a small data set (12 values) and so these plots hold a limited amount of information, but do not appear inconsistent with the assumptions of equal variance and Normal distribution made for the model deviations.

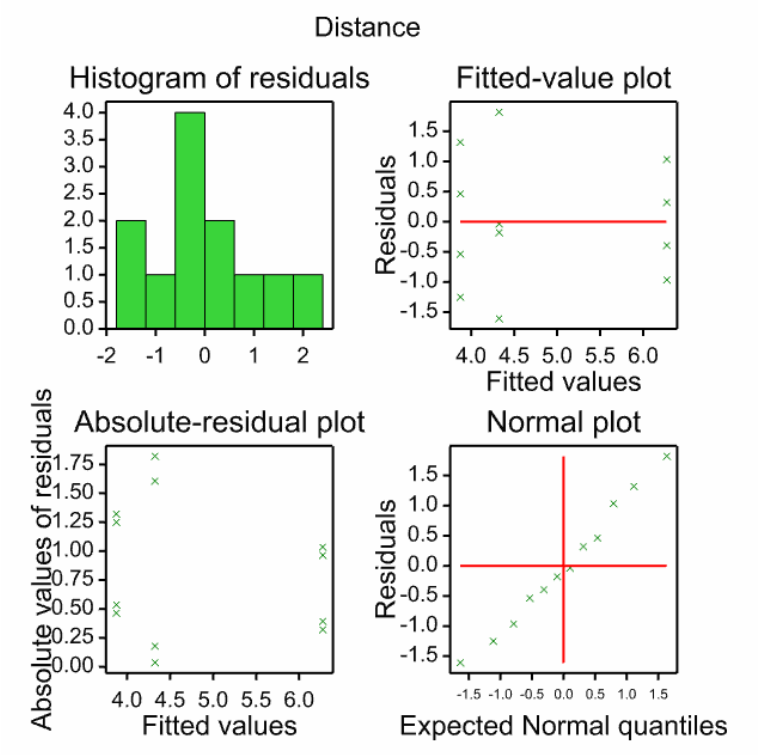

Figure S8.6.1. Residual plots for standardized residuals from analysis of distance travelled.

Table S8.6.1. ANOVA table for one-way model for distance travelled by nematodes.

Source of variation	df	Sum of squares	Mean square	Variance ratio	*P*
Treatment	2	13.020	6.510	9.960	0.005
Residual	9	5.882	0.654		
Total	11	18.902			

Table 8.6.1 shows the ANOVA table from this analysis. The variance ratio for the treatment term ($F_{2,9}$ = 9.960, P = 0.005) gives strong evidence for differences between the population means for the three solutions.

We have been asked to use contrasts to answer two specific questions: whether the addition of lectins affects nematode movement, and whether there is any difference between the two lectin solutions. We can write down the model in mathematical terms as

$$y_{ij} = \mu + \tau_i + e_{ij} \ ,$$

where y_{ij} is the distance travelled in the jth dish in the ith treatment group (i=1 for CONA, i=2 for GNA and i=3 for PBS), μ is the overall population mean, τ_i is the difference between the population mean

of the *i*th treatment group and the overall population mean, and e_{ij} is the model deviation for the *j*th dish in the *i*th treatment group. We can then translate the first question into the null hypothesis

$$\tfrac{1}{2}(\tau_1 + \tau_2) = \tau_3 \ ,$$

ie. the population average over the lectin treatments is equal to that of the control treatment (PBS with *i*=3). We rewrite this as a contrast as

$$(0.5 \times \tau_1) + (0.5 \times \tau_2) - \tau_3 \ = \ 0 \, .$$

.

The contrast coefficients corresponding to the first question are therefore 0.5, 0.5, -1. The second question is a straight contrast between the first and second treatments (CONA and GNA), ie.

$$\tau_1 - \tau_2 \ = \ 0 \, ,$$

with contrast coefficients 1, -1, 0. The ANOVA table with the treatment SS partitioned by these factors is shown in Table 8.6.2. There is strong evidence of a difference in population mean distance travelled between treatments with lectins and the control PBS solution (F^{LvP} = 19.300 with 1 and 9 df, *P* = 0.002), but no evidence of any difference in population means between the two lectin solutions (F^{CvG} = 0.620, *P* = 0.451). The estimated contrast values are shown in Table S8.6.3; note that the observed significance level of the t-ratios exactly matches that of the variance ratios in the ANOVA table. The estimated population mean distance travelled by nematodes in the lectin solution was 2.18 units less than that in the standard (PBS) solution.

Note that the same results can be obtained by using factors to represent the contrasts. The first contrast is equivalent to a factor with level 1 for CONA and GNA, and level 2 for PBS. We will call this factor Control. Fitting the nested explanatory component [1] + Control/Treatment first fits the lectin vs control contrasts, via the Control factor. The only comparison left is that between the two lectin treatments, which is therefore represented by the Control.Treatment term.

Table S8.6.2. ANOVA table for distance travelled with treatment SS partitioned by contrasts.

Source of variation	df	Sum of squares	Mean square	Variance ratio	*P*
Treatment	2	13.020	6.510	9.960	0.005
Lectins vs PBS	1	12.615	12.615	F^{LvP} = 19.300	0.002
CONA vs GNA	1	0.405	0.405	F^{CvG} = 0.620	0.451
Residual	9	5.882	0.654		
Total	11	18.902			

Table S8.6.3. Predicted contrasts with SE.

Contrast	Description	Estimate	SE	t-ratio	*P*
$(0.5 \times \tau_1) + (0.5 \times \tau_2) - \tau_3$	Lectins vs PBS	-2.18	0.495	-4.393	0.002
$\tau_1 - \tau_2$	CONA vs GNA	-0.45	0.572	-0.787	0.451