Solution to Exercise 9.2 (Version 1, 11/7/15)

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Exercise 9.2 (Data: courtesy E. Wright, Rothamsted Research)

An experiment used three incubators to compare growth of fungal colonies of *Metarhizium anisopliae* at three temperatures (23, 30 and 35°C; Wright, 2013). Replication of temperatures was achieved by repeating the experiment on three occasions. Temperatures were allocated to incubators according to a 3×3 Latin square, so each incubator ran once at each temperature. Small fungal plugs were placed in Petri dishes and three dishes were placed in each incubator on each occasion. The sizes of the fungal colonies were recorded after four days. The dish numbers (*ID*), structural factors (Incubator, Occasion and Dish), explanatory factor (Temperature) and size measurements (variate *Size*) are given in file SIZE.DAT. Write down the structural component of the model for the colony sizes. Analyse the data and state your conclusions. What can you say about the effect of temperature on the growth of these fungal colonies?

Data 9.2 (SIZE.DAT)

Sizes (S) of *M. anisopliae* fungal colonies grown in dishes (D) at different temperatures (T, °C) in an experiment using three incubators (I) on three occasions (O):

ID	I	O	D	T	S	ID	I	O	D	T	S
1	1	1	1	23	13.15	16	2	3	1	35	3.95
2	1	1	2	23	13.40	17	2	3	2	35	3.75
3	1	1	3	23	13.90	18	2	3	3	35	4.05
4	1	2	1	35	3.55	19	3	1	1	35	4.25
5	1	2	2	35	3.65	20	3	1	2	35	4.30
6	1	2	3	35	3.65	21	3	1	3	35	4.20
7	1	3	1	30	13.80	22	3	2	1	30	13.70
8	1	3	2	30	14.45	23	3	2	2	30	13.15
9	1	3	3	30	14.35	24	3	2	3	30	13.10
10	2	1	1	30	13.35	25	3	3	1	23	13.75
11	2	1	2	30	13.70	26	3	3	2	23	12.55
12	2	1	3	30	14.30	27	3	3	3	23	13.05
13	2	2	1	23	13.15						
14	2	2	2	23	12.55						
15	2	2	3	23	12.55						

Source: Wright, E.L. 2013. *The effect of pathogens on honeybee learning and foraging behaviour*. PhD thesis, University of Warwick.

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As the same incubators are used on each occasion, these two factors are crossed. Dishes are nested within incubator on each occasion, as there is no relationship between dishes used in different incubators or on different occasions. We can draw this structure, including the temperature allocation, as in Figure S9.2.1. As stated in the question, this is a Latin Square design with each temperature being allocated once to each of the incubators and once within each replicate (occasion). The dishes are pseudo-replicates with respect to the temperature treatments, but this will not be a problem provided that we give the correct specification of the structural component.
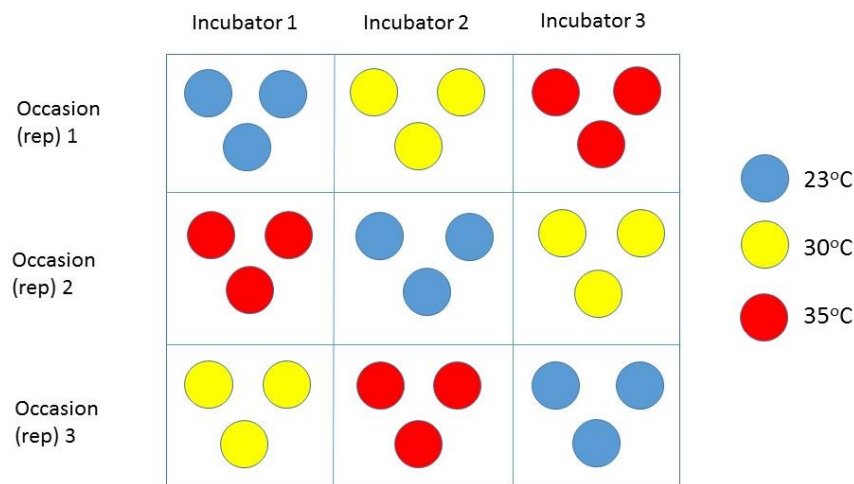

Figure 9.2.1. Allocation of temperatures to dishes in 3 incubators on 3 separate occasions.

Hence the structural component of the model for colony sizes can be written as

Structural component: (Occasion * Incubator) / Dish

There is a single explanatory factor, Temperature, which is applied to whole incubators within occasions, and the response variate *Size*, the measure of colony size on each dish. The full model can therefore be written as

Response variate: *Size*
Structural component: (Occasion * Incubator) / Dish
Explanatory component: [1] + Temperature

A composite set of residual plots from this model are given in Figure S9.2.2. The fitted values and absolute residuals plots give strong evidence of variance heterogeneity and so we apply the logarithm (base 10) transformation to the size variate, ie.

$$log10Size = \log_{10}(Size)$$

The model for the transformed data is written

Response variate: *log10Size*
Structural component: (Occasion * Incubator) / Dish
Explanatory component: [1] + Temperature

A composite set of residual plots from this model are in Figure S9.2.3. There is still a slight suggestion of variance heterogeneity in the fitted value and absolute residual plots, but no other transformation could be found that improved upon this. Overall, in the absence of alternatives, we judge these plots to be acceptable and move on to examine the ANOVA table.
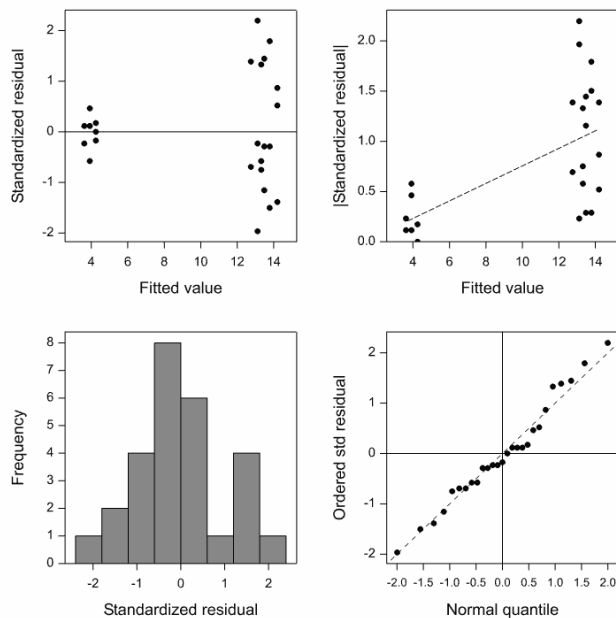

Figure S9.2.2. Composite set of residual plots using standardized residuals from ANOVA of fungal colony sizes.
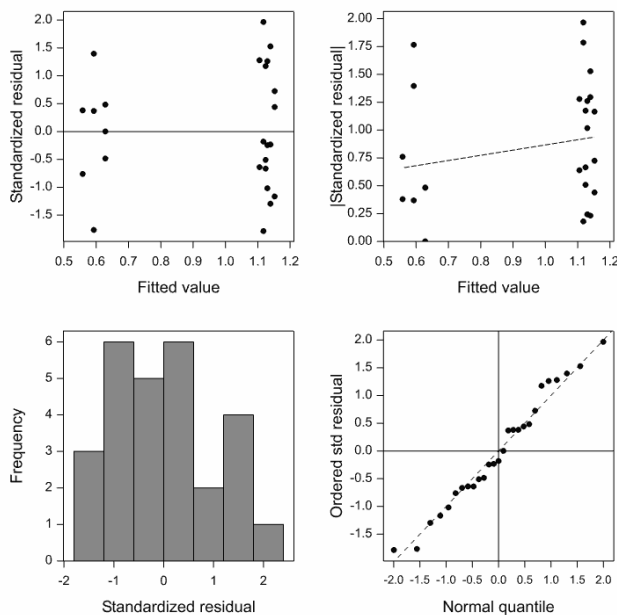

Figure S9.2.3. Composite set of residual plots using standardized residuals from ANOVA of logged fungal colony sizes.

The ANOVA table for this model is in Table S9.2.1. The SS for the temperature treatment appears in the Occasion.Incubator stratum, as we want. This means that the Temperature variance ratio is tested using the correct (but rather small) residual df rather than being artificially inflated by df borrowed from the pseudo-replication of dishes. Despite the small number of residual df in this stratum, there is strong evidence that population mean colony sizes differ among temperatures ($F_{2,2} = 679.34$, $P = 0.001$). Predicted population means are given in Table S9.2.2 on the transformed scale, together with back-transformed means, and are plotted with the data on the log10-scale in Figure S9.2.4. Predicted population mean colony sizes are similar at 23 and 30°C, but much smaller at 35°C.

Table S9.2.1 Multi-stratum ANOVA table for logged fungal colony sizes.

Source of variation	df	Sum of squares	Mean square	Variance ratio	P
Occasion stratum	2	0.00623	0.00311	2.462	0.289
Incubator stratum	2	0.00066	0.00033	0.263	0.792
Occasion.Incubator stratum					
Temperature	2	1.71893	0.85947	679.339	0.001
Residual	2	0.00253	0.00127	7.550	0.004
Occasion.Incubator.Dish stratum					
Residual	18	0.00302	0.00017		
Total	26	1.73137			

Table S9.2.2 Predicted population mean $\log_{10}$(colony size) with back-transformed values. SED = 0.01677, LSD = 0.07214 (for $\alpha_s = 0.05$), 2 df. SED and LSD apply to $\log_{10}$-scale only.

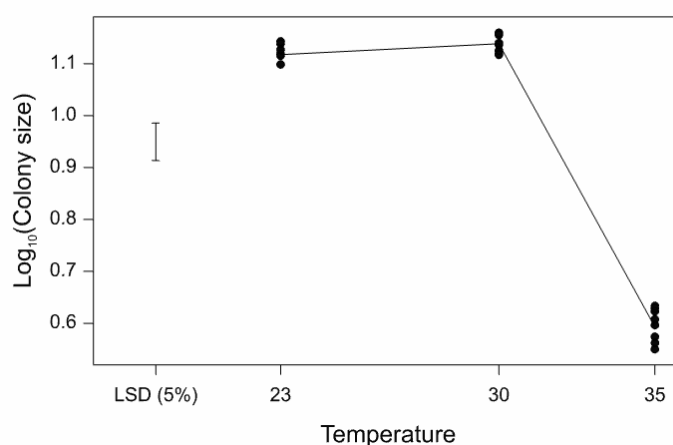|	Temperature, °C		
	23	30	35
Mean ($\log_{10}$ scale)	1.1175	1.1386	0.5931
Back-transformed mean	13.11	13.76	3.92

Figure S9.2.4. Predicted population mean $\log_{10}$(colony size) (joined by lines) at three temperatures (°C), with LSD (for $\alpha_s = 0.05$) and logged data (●).