Biostats VHM 802 Course
Winter 2015, Atlantic Veterinary College, PEI
Henrik Stryhn

Solution to Additional Exercise 7.7

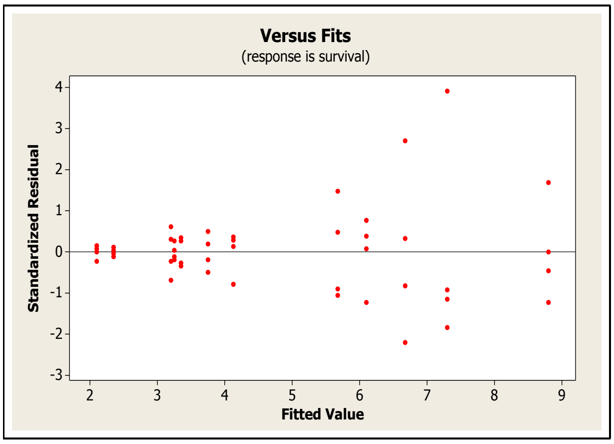
The description of the study suggests that the experiment was carried out in a completely randomized design with the two factors treatment and poison. We could describe it as a 3×4-design with replication (4 replicates per factor combination). The most natural model is therefore the full factorial model for the two factors. Denote by y_{ijk} the survival time of animal k subjected to poison i and treatment j, where $i = I, II, III$ and $j =$A,B,C,D and $k = 1, 2, 3, 4$. The model then takes the form,

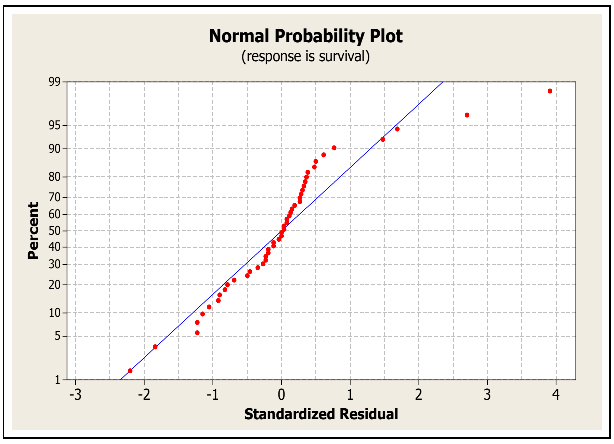
$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

where the α_i's and β_j's correspond to the main effect of poisons and treatments, respectively, the $(\alpha\beta)_{ij}$'s correspond to the interactions and ε_{ijk}'s are i.i.d. and $\sim \mathrm{N}(0, \sigma^2)$.

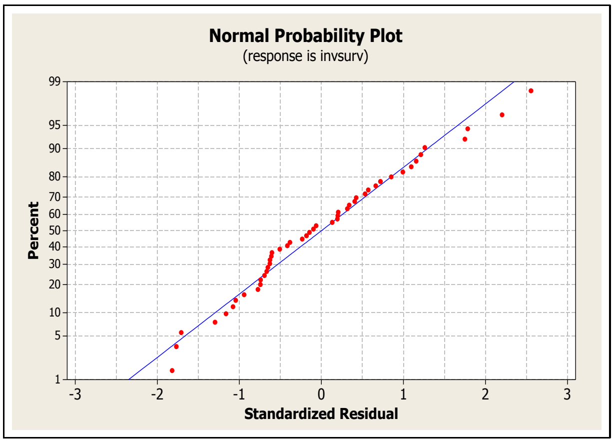
1. Transformation of the outcome

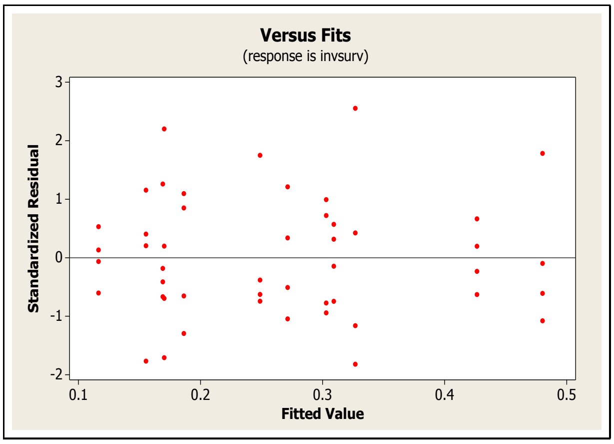
The residual plots (below) for the model described above look pretty bad. There is a strong cone (or fan) shape in the residuals versus fitted values plot, indicating heteroscedasticity whereby the variance increases with the mean. The probability plot does not look good either, mostly due to several large positive residuals. Transformation of the outcome is suggested as a way to meet the model assumptions.





All observations are positive so it is natural to try a power transformation. One can either proceed by trial and error, or estimate the optimal power within the Box-Cox class of transformations. Evaluating a grid of transformations, e.g. using R software, indicates the optimal λ to be between -0.5 and -1. More specifically, a Box-Cox transformation analysis in Stata or Minitab (`General Regression` menu) gives the optimal $\hat{\lambda} = -0.839$ with approximate 95% confidence intervals of $(-1.308, -0.369)$ and $(-1.315, -0.375)$, respectively. The closest "nice" value to the estimate is -1, so we try a reciprocal (inverse) transformation: $z_{ijk} = 1/y_{ijk}$. Both residual plots (below) are strongly improved and quite acceptable. The inverse square-root transformation ($\lambda = -\frac{1}{2}$) also gives acceptable residual plots.





2. Outliers

After transformation, the most extreme residuals are not very extreme. For the reciprocal transformation, the most extreme standardised residual has a value of 2.56 (observation 20), corresponding to a deletion residual of 2.786. The P-value for the outlier test based on standardised residuals equals $2 \cdot 48 \cdot P(t_{35} > 2.786) = 96 \cdot 0.043 = 0.41$ and is nowhere near significant. (At the inverse square-root scale one obtains a deletion residual of 2.62 and $P = 0.62$.) We conclude that the data contain no outlying observations. Note that the large residuals in the residual plots for untransformed data are therefore most likely a result of the right-skewedness of the error distribution. After the transformation, these are no longer of concern.

3. Treatment and poison effects

The ANOVA table below shows that there is no indication of an interaction between poison and treatment effects, and that the main effects of both factors are strongly significant ($P < 0.0005$). As the design is balanced, there is no need to refit the model without the interaction. The listing includes means with model-based standard errors as well as Bonferroni-adjusted pairwise comparisons between treatments.

```
Analysis of Variance for invsurv, using Adjusted SS for Tests

Source         DF    Seq SS    Adj SS    Adj MS      F      P
poison          2  0.344441  0.344441  0.172221  72.45  0.000
treat           3  0.194706  0.194706  0.064902  27.30  0.000
poison*treat    6  0.014743  0.014743  0.002457   1.03  0.420
Error          36  0.085571  0.085571  0.002377
Total          47  0.639461

S = 0.0487541   R-Sq = 86.62%   R-Sq(adj) = 82.53%
```

```
Least Squares Means for invsurv

poison    Mean  SE Mean
I       0.1801  0.01219
II      0.2309  0.01219
III     0.3797  0.01219
treat
A       0.3519  0.01407
B       0.1915  0.01407
C       0.2947  0.01407
D       0.2161  0.01407

Bonferroni Simultaneous Tests
Response Variable invsurv
All Pairwise Comparisons among Levels of treat

treat = A  subtracted from:
       Difference       SE of            Adjusted
treat    of Means  Difference  T-Value    P-Value
B         -0.1604     0.01990   -8.060     0.0000
C         -0.0572     0.01990   -2.875     0.0405
D         -0.1358     0.01990   -6.825     0.0000

treat = B  subtracted from:
       Difference       SE of            Adjusted
treat    of Means  Difference  T-Value    P-Value
C         0.10322     0.01990    5.186     0.0001
D         0.02460     0.01990    1.236     1.0000

treat = C  subtracted from:
       Difference       SE of            Adjusted
treat    of Means  Difference  T-Value    P-Value
D        -0.07862     0.01990   -3.950     0.0021
```

The means for the poisons show poison I to have the lowest estimated survival time (on transformed scale), whereas the means for poisons II and III are higher. As the reciprocal transformation reverses the order of observations, this means that estimated survival times are largest with poison I and lower with the other two poisons. To assess any difference between poison types (III versus I and II), we set up the contrast,

$$w_1 = \mu_{III} - \tfrac{1}{2}(\mu_I + \mu_{II}), \quad \text{with } \mu_i = \mu + \alpha_i.$$

We estimate the μ_i's by the corresponding means, so that $\hat{w}_1 = 0.3797 - \frac{1}{2}(0.1801 + 0.2309) = 0.1742$. Furthermore, we estimate $\mathrm{Var}(\hat{w}_1)$ (the squared $\mathrm{SE}(\hat{w}_1)$) by $\mathrm{MSE}(1^2 + (-\frac{1}{2})^2 + (-\frac{1}{2})^2)/16 = 0.000223 = 0.0149^2$. Finally, we compute the t-statistic for the hypothesis H_0: $w_1 = 0$ as $t = \hat{w}_1/\mathrm{SE}(\hat{w}_1) = 11.7$, which is very clearly significant in a $t(36)$-distribution. There is certainly a difference between poison types.

The remaining, orthogonal contrast is $w_2 = \mu_I - \mu_{II}$, for which we obtain $\hat{w}_2 = 0.051$ and $\mathrm{SE}(\hat{w}_2) = 0.017$. Thus, the t-statistic for H_0: $w_2 = 0$ is $t = 2.95$, with a $P = 0.003$. We conclude that there is also a clear difference between the 2 poisons of same type, and therefore clear differences among all 3 poisons, with poison III being the most effective and poison I being the least effective.

As no special relations exist among the treatments, we carry out all pairwise comparisons, using the Bonferroni method to adjust for multiple comparisons. The table in the Minitab listing shows that only treatments B and D are not significantly different. These two treatments also have the lowest means on transformed scale, thus corresponding to longest survival times.

4. Estimated survival times

For animals subjected to poison III, we compute a mean with its 95% confidence interval as follows:

- from the listing of Least square means: $\hat{\mu}_{III} = 0.3797$, $\mathrm{SE}(\hat{\mu}_{III}) = 0.01219$,
- 95% CI for μ_{III}: $0.3797 \pm t(36, .975) \cdot 0.01219 = 0.3797 \pm 0.0247 = (0.3550, 0.4044)$,
- 95% CI for $1/\mu_{III}$: $(1/0.4044, 1/0.355) = (2.47, 2.82)$,

where we used $t(36, .975) = 2.028$. Note that the backtransformed value, $1/\hat{\mu}_{III} = 1/0.3797 = 2.63$, and its confidence interval are for the median (and not the mean) survival time.

Estimates of the survival time at a poison by treatment combination can be based either on the full model including the interaction or the reduced, additive model. The (least squares) means for the combined factor `poison*treat` includes the value 0.4803 with a standard error of 0.02438 ($= 0.0487541/\sqrt{4}$) for poison III and treatment A, giving the 95% confidence interval (0.431,0.530) on transformed scale and (1.89,2.32) on original scale, together with the estimate (median) of $1/0.4803 = 2.082$. However, estimation in the full model seems inconsistent with the clearly non-significant interaction and will for the same reason be less precise than using the additive model. Note that the least squares means for poisons are the same regardless of whether the interaction is included or not, and the change in SE only reflects the pooling of the interaction into error in the additive model ($\mathrm{SE}(\hat{\mu}_{III}) = 0.01222$).

In the absence of an interaction between poisons and treatments (i.e., the additive model), the estimated level (on transformed scale) for poison III and treatment A is computed as (shown for example in GO Display 8.2):

$$
\begin{aligned}
\hat{\mu} + \hat{\alpha}_3 + \hat{\beta}_1 &= \bar{y}_{3\cdot\cdot} + \bar{y}_{\cdot 1\cdot} - \bar{y}_{\cdot\cdot\cdot} \\
&= 0.3797 + 0.3519 - 0.2636 = 0.468.
\end{aligned}
$$

Using statistical software, this value can be obtained as the fitted value for observations with poison III and treatment A in the additive model (after refitting the model without the interaction). If your statistical software offers the standard error (not prediction error!) on predicted values, this is one way to get it (in a model without the interaction term). Alternatively, your software may offer estimation commands that can estimate this linear combination of parameters with a standard error. Among these, Minitab will only do the former (requesting a prediction interval in the `Options` submenu of the `General Linear Model` menu). This can be done also in Stata (using the `predict` command), or one may use the `lincom` command to get inference for the linear combination. Either way, we get $\mathrm{SE}(\hat{\mu} + \hat{\alpha}_3 + \hat{\beta}_1) = 0.0173$ and a 95% CI of $(0.433, 0.503)$. At original scale this corresponds to an estimated median of $1/0.468 = 2.14$ and a 95% CI of (1.99,2.31).