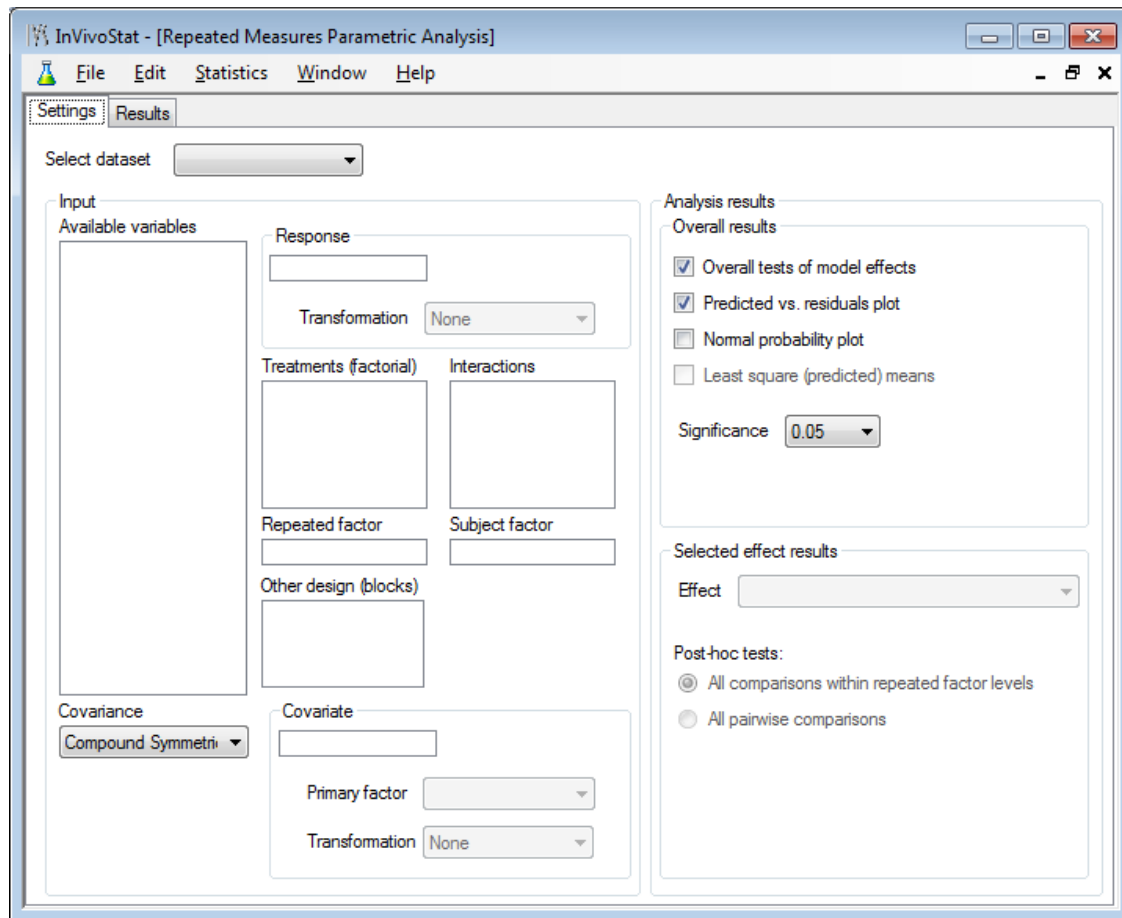


The Repeated Measures Parametric Analysis module in SilveR is available from the Statistics drop-down menu entitled “Repeated Measures Parametric Analysis”. The user interface is:



The Repeated Measures Parametric Analysis module performs a Linear Mixed Model analysis. It should be used when the animals have been measured repeatedly. This module allows the user to fit multiple treatment (factorial) factors, other design (blocks) factors and a single covariate. All interactions involving the treatment factors are included in the statistical model but no interactions involving the blocking factors are included.

1 Setting up the model

Once the dataset has been opened, the user can select the variables for the analysis by dragging and dropping them from the “Available variables” list into the “Response”, “Treatments (factorial)”, “Other design (blocks)”, “Covariate”, “Repeated” and “Subject” boxes.

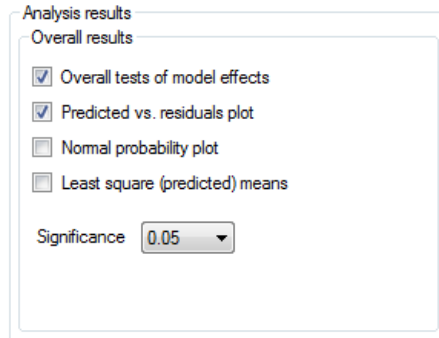
Once selected, the user has the option of applying a transformation to the response, either \log_{10} , \log_e , square root, arcsine or rank. If selected, the covariate will be transformed using the same transformation applied to the response variable, unless otherwise specified by the user.

If a covariate is selected, then the user has the option of selecting the “Primary factor”. This factor is used to categorise the scatterplot (produced in the output). The Primary factor should be one of the factors of interest to the experimenter.

Finally the user can select a covariance structure to model the within animal observations. The default is the compound symmetric structure (all observations within animal are equally correlated regardless of the “distance” apart). Other options include autoregressive (recommended if the time points are equally spaced) or unstructured (recommended if the sample size is not too small).

2 Selecting the analysis options

There are several results from the General Linear Mixed Model analysis that are available to the user. These are selected before running the analysis. These include the overall tests:



1) Overall tests of model effects

Produces overall tests of the effects of the factors and also any interactions present in the statistical model.

2) Predicted vs. residuals plot

Allows the user to check the variance assumption of the parametric analysis.

3) Normal probability plot

Allows the user to check the normality assumption of the parametric analysis.

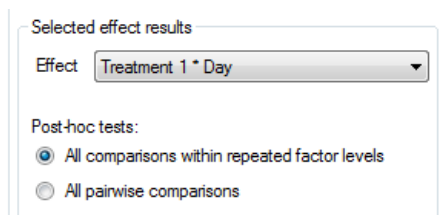
4) Least square (predicted) means

The repeated measures module produces a plot of the predicted means and confidence limits of either the treatment factor levels over time (if only a single treatment factor is selected) or the levels of the highest order factorial treatment interaction over time (for multiple treatment factors).

5) Significance level

The default is 5%, although this can be changed.

The user then has various options available:



6) Selected effect

This is the effect that the user is interested in comparing the levels of. Any treatment factor (over time), or treatment factor interaction (over time) effect may be selected.

7) All pairwise tests

Produces all pairwise comparisons between the levels of the Selected effect at each time point. The user can either select all possible pairwise tests, or limit the output to consist of only the “within time” comparisons.

3 Output Results

Response, covariance structure and covariate

SilveR identifies the response being analysed, the covariance structure used and also the covariate (if one is selected). This section also describes any transformations that have been applied.

Categorised case profile of the raw data

SilveR produces a categorised case profile of the raw data (see graphics module). The plot is categorised by either the treatment factor (if only one is selected) or the levels of the highest order treatment interaction (if multiple treatment factors are selected). This plot should be used to identify possible outliers.

Covariate plot of the raw data

When fitting a covariate in a statistical analysis, certain assumptions are made. This plot allows the user to test these assumptions. Underneath the plot is a list of the additional assumptions being made and also advice on how the plot can be used to test them. The scatterplot is categorised by the Primary factor at each level of the repeated factor.

Overall table of effects

The ANOVA-style table gives tests of the overall effects of the factors and any interactions present. Below the table any statistically significant effects are listed.

Diagnostic plots

If requested SilveR produces the predicted vs. residuals plot and the normal probability plot. The residuals plotted on the predicted vs. residuals plot are the Standardized residuals as these can provide a test for outliers. Any observation with a residual greater (or less than) 3 could be considered an outlier.

Plot of the predicted means

SilveR produces a plot and table of the predicted means of either the treatment factor (if only one treatment is included in the statistical model) or the highest order interaction (if multiple treatment factors are selected). Also included are confidence intervals around the means.

All pairwise tests

SilveR produces a table of all pairwise comparisons between the levels of the Selected effect over time. As well as the size of the difference between the predicted means, SilveR also provides p-values to test the significance of these effects. A list of statistically significant comparisons is given below the table.

References

Finally a list of references for the methods applied in the analysis is given.

4 Sample output

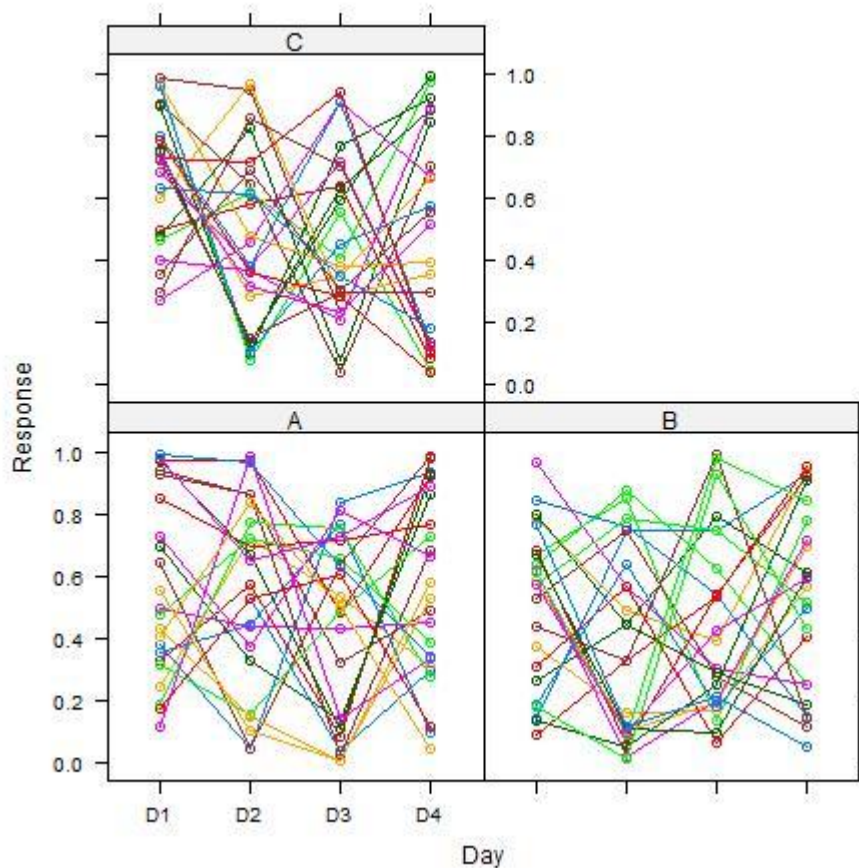
SilveR Repeated Measures Parametric Analysis

Response, covariate and covariance structure

The Response response is currently being analysed by the Repeated Measures Parametric Analysis module, with Covariate fitted as a covariate.

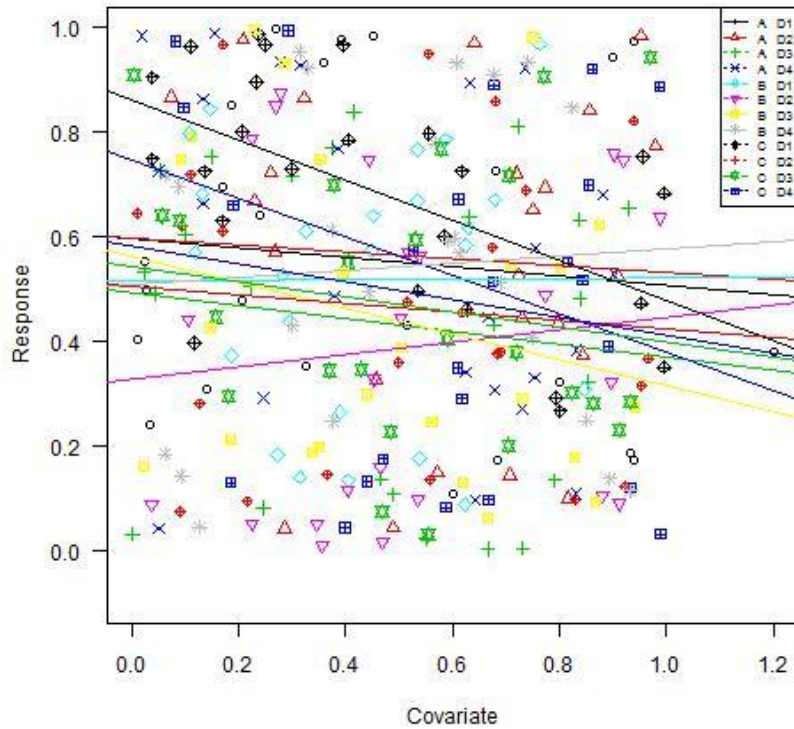
The Linear Mixed Model analysis is using the compound symmetric covariance structure to model the within animal correlations. When using this structure you are assuming sphericity and also that the variability of responses is the same at each level of Day, see Pinherio and Bates (2002). These assumptions may not be valid in practice.

Categorised case profiles plot of the raw data



Tip: Use this plot to identify possible outlier (animals and individual observations) and decide on suitable summary measures.

Covariate plot of the raw data



Tip: Is it worth fitting the covariate? You should consider the following:

- a) Is there a relationship between the response and the covariate?... It is only worth fitting the covariate if there is a strong positive (or negative) relationship between them. The lines on the plot should not be horizontal.
 - b) Is the relationship similar for all treatments?... The lines on the plot should be approximately parallel.
 - c) Is the covariate influenced by the treatment?... We assume the covariate is not influenced by the treatment so there should be no separation of the treatment groups along the x-axis on the plot.
- These issues are discussed in more detail in Morris (1999).

Overall tests of model effects

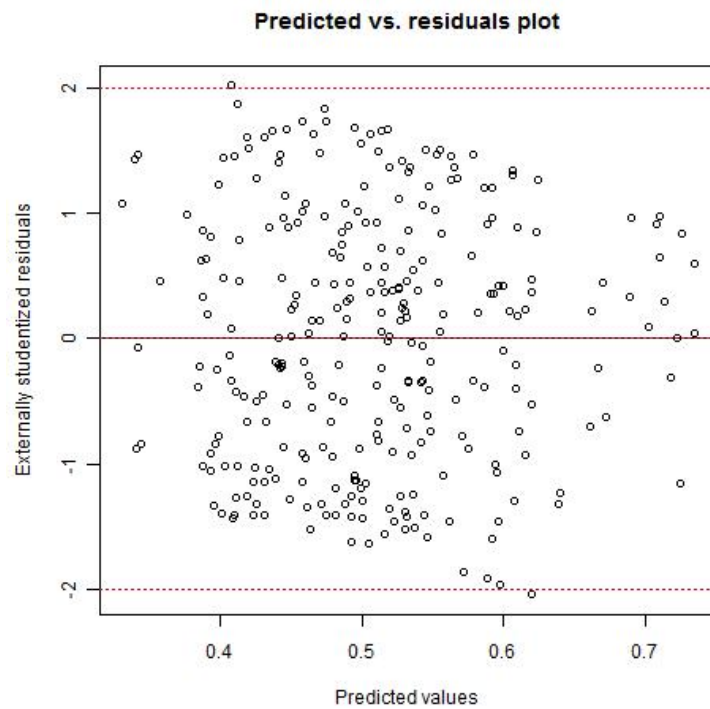
	numDF	denDF	F-value	p-value
Covariate	1	206	4.94	0.0273
Treatment 1	2	69	1.62	0.2057
Day	3	206	2.83	0.0393
Treatment 1:Day	6	206	1.22	0.2995

Comment: The overall tests in this table are likelihood ratio tests.

Conclusion: At the 5% level there is a statistically significant overall difference between the levels of Covariate, Day.

Tip: While it is a good idea to consider the overall tests in the above table, we should not rely on them when deciding whether or not to make pairwise comparisons between the factor levels.

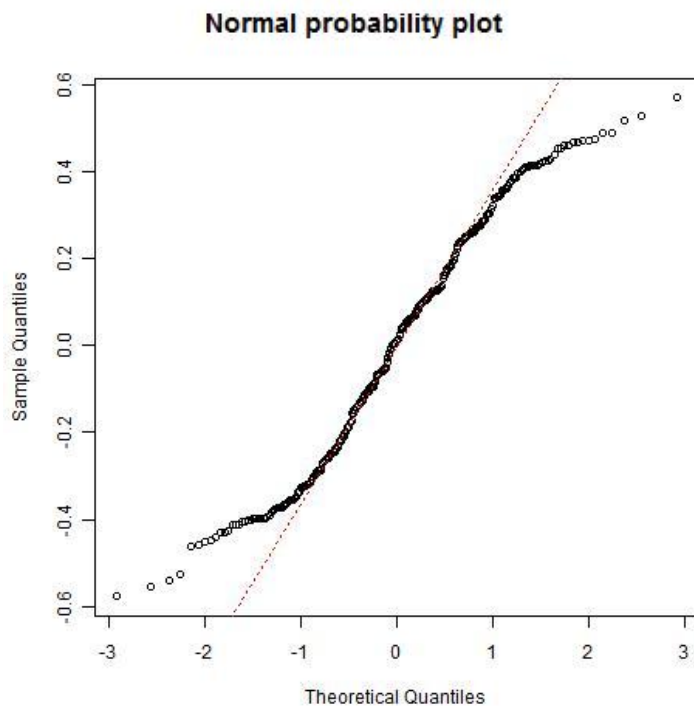
Diagnostic plots



Tip: On this plot look to see if the spread of the points increases as the predicted values increase. If so the response may need transforming.

Tip: Any observation with a studentized residual less than -3 or greater than 3 (SD) should be investigated as a possible outlier.

Comment: The residuals at level i are obtained by subtracting the fitted levels at that level from the response vector and dividing by the estimated within-group standard error.



Tip: Check that the points lie along the dotted line. If not then the data may be non-normally distributed.

Plot of the predicted means with 95% confidence intervals

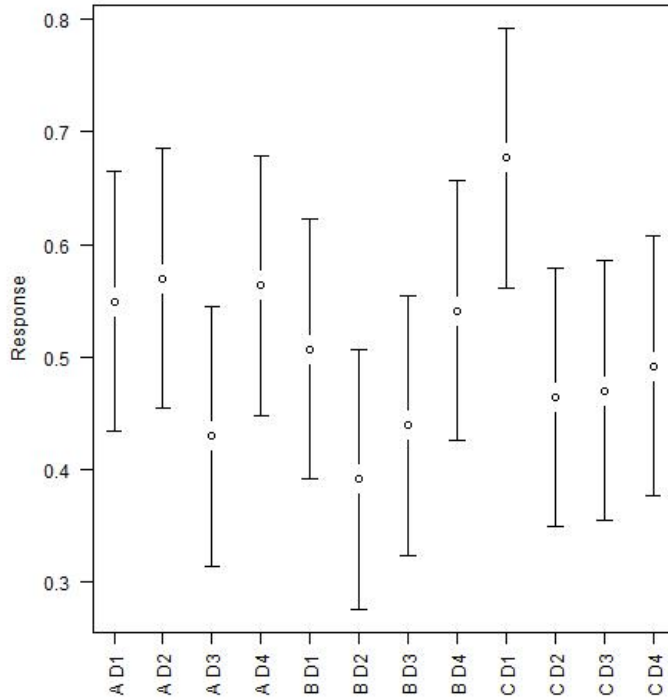


Table of the predicted means with 95% confidence intervals

Level	Mean	Lower 95% CI	Upper 95% CI
A D1	0.55	0.43	0.66
A D2	0.57	0.45	0.69
A D3	0.43	0.31	0.54
A D4	0.56	0.45	0.68
B D1	0.51	0.39	0.62
B D2	0.39	0.28	0.51
B D3	0.44	0.32	0.55
B D4	0.54	0.43	0.66
C D1	0.68	0.56	0.79
C D2	0.46	0.35	0.58
C D3	0.47	0.35	0.59
C D4	0.49	0.38	0.61

Pairwise comparisons within the levels of the repeated factor, without adjustment for multiplicity

Comparison	Difference	Lower 95% CI	Upper 95% CI	SEM	p-value
D1 : B vs. A	-0.04	-0.20	0.12	0.083	0.612
D1 : C vs. A	0.13	-0.04	0.29	0.083	0.124
D2 : B vs. A	-0.18	-0.34	-0.02	0.083	0.032
D2 : C vs. A	-0.11	-0.27	0.06	0.083	0.203
D3 : B vs. A	0.01	-0.15	0.17	0.083	0.908
D3 : C vs. A	0.04	-0.12	0.20	0.083	0.624
D4 : B vs. A	-0.02	-0.18	0.14	0.083	0.789
D4 : C vs. A	-0.07	-0.23	0.09	0.083	0.391
D1 : C vs. B	0.17	0.01	0.33	0.083	0.041
D2 : C vs. B	0.07	-0.09	0.24	0.083	0.376
D3 : C vs. B	0.03	-0.13	0.19	0.083	0.707
D4 : C vs. B	-0.05	-0.21	0.11	0.083	0.554

Warning: As these tests are not adjusted for multiplicity there is a risk of false positive results. Only use the pairwise tests you planned to make a-priori, these are the so called Planned Comparisons, see Snedecor and Cochran (1989). No options are available in this module to make multiple comparison adjustments. If you wish to apply a multiple comparison adjustment to these results then use the p-value adjustment module.

Conclusion: The following pairwise tests are statistically significantly different at the 5% level: D2 : B vs. A, D1 : C vs. B.

Analysis description

The data were analysed using a 2-way repeated measures Linear Mixed Model approach, with treatment factor Treatment 1, repeated factor Day and Covariate as the covariate.

The compound symmetric covariance structure is used to model the within animal correlations. This makes the assumption that the variability of the responses is the same at each level of Day and the correlation between responses from any pair of levels of Day is the same.

A full description of Linear Mixed Model theory, including information on the R nlme package used by SilveR, can be found in Venables and Ripley (2003) and Pinherio and Bates (2002).

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