# Using asreml and asremlPlus for the Ladybird example from Welham et al. (2014)

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## Introduction

This vignette shows how to use asremlPlus (Brien, 2020a), and dae (Brien, 2020b), for exploring and presenting predictions from a linear mixed model analysis, the predictions having been produced using asreml (Butler et al., 2018). Here, asremlPlus, dae and asreml are packages for the R Statistical Computing environment (R Core Team, 2020).

The context is a three-factor factorial experiment on ladybirds (Welham et al., 2014, Example 8.2) that aims to answer the question "Will ladybirds transfer fungus to aphids on plants?" The experiment consists of 2 runs of 36 containers, each with a plant and aphids. There are three factors that results in 12 treatments: Host plant (beans, trefoil), infected Cadavers (5, 10, 20), Ladybird (-, +). Ther are randomized to the containers within a run so that each is replicated 3 times within a run. The respose to be analysed is the logit of the proportion of live aphids that were infected.

## Initialize

```
library(knitr)
opts_chunk$set("tidy" = FALSE, comment = NA)
suppressMessages(library(asreml))
packageVersion("asreml")

## [1] '4.1.0.110'
suppressMessages(library(asremlPlus))
packageVersion("asremlPlus")

## [1] '4.2.14'
suppressMessages(library(dae))
packageVersion("dae")

## [1] '3.1.22'
options(width = 95, show.signif.stars = FALSE)
```

## Get data available in asremlPlus

```
data("Ladybird.dat")
```

## Do an ANOVA of logits

Error: Run

Df Sum Sq Mean Sq F value Pr(>F)

Residuals 1 0.06766 0.06766

Error: Run:Plant

	$\mathtt{Df}$	Sum Sq	Mean Sq	${\tt F} \ {\tt value}$	Pr(>F)
Host	1	13.599	13.599	59.172	1.82e-10
Cadavers	2	17.027	8.514	37.044	3.78e-11
Ladybird	1	11.091	11.091	48.257	3.33e-09
Host:Cadavers	2	0.308	0.154	0.670	0.5158
Host:Ladybird	1	0.228	0.228	0.992	0.3234
Cadavers:Ladybird	2	1.735	0.867	3.774	0.0287
Host:Cadavers:Ladybird	2	0.200	0.100	0.435	0.6493
Residuals	59	13.560	0.230		

The anova table gives the F-tests for the three-factor effects and interactions. Note the Residuals Mean Sq value for Run:Plant of 0.230. Also, it is clear that the Run component is negative, given that the Residuals Mean Sq value for Run is less than that for Run:Plant; it is (0.06766 - 0.230) / 36). From the table it is seen that the only significant interaction is Cadavers:Ladybird and that the Host main effect is significant.

## Use asreml to analyse the logits

## Mixed model analysis of logits

Model fitted using the gamma parameterization.

ASReml 4.1.0 Fri Feb 14 20:34:59 2020

	LogLik	Sigma2	DF	wall	cpu	
1	3.07130	0.226239	60	20:34:59	0.0 (	1 restrained)
2	3.64260	0.226912	60	20:34:59	0.0 (	1 restrained)
3	3.71250	0.227106	60	20:34:59	0.0 (	1 restrained)
4	3.71721	0.227121	60	20:34:59	0.0 (	1 restrained)
5	3.71751	0.227122	60	20:34:59	0.0 (	1 restrained)

Warning in asreml(logitP  $\sim$  Host \* Cadavers \* Ladybird, random =  $\sim$ Run, residual =  $\sim$ Run:Plant, : Some components changed by more than 1% on the last iteration.

```
summary(m)$varcomp
```

```
component std.error z.ratio bound %ch
Run 2.298309e-08 NA NA B 93.7
Run:Plant!R 2.271216e-01 0.04156985 5.463612 P 0.0
```

As expected the Run component is bound (B) at approximately zero. This results in a change in the estimate of the residual variance to 0.227. To allow for a negative estimate we will unconstrain the Run component. As Littell et al. (2006, p.150) say

if you do not set the negative variance component estimate to zero, but allow it to remain negative, you get better control over Type I error and, for cases of negative wholeplot error variance estimates, greater power. Therefore, this is the recommended procedure.

## Unconstrain Reps to make the analysis equivalent to ANOVA

```
m <- setvarianceterms(m$call, terms = "Run", bounds = "U")
Model fitted using the gamma parameterization.
ASReml 4.1.0 Fri Feb 14 20:34:59 2020
                        Sigma2
          LogLik
                                           wall
                                                    cpu
 1
         3.07130
                      0.226239
                                    60 20:34:59
                                                    0.0 (1 restrained)
 2
         3.64260
                      0.226912
                                    60 20:34:59
                                                    0.0 (1 restrained)
 3
                                                    0.0 (1 restrained)
         3.80283
                      0.227454
                                    60 20:34:59
 4
         3.83949
                      0.233418
                                    60 20:34:59
                                                    0.0
 5
                                    60 20:34:59
         3.95568
                      0.230971
                                                    0.0
 6
         3.97333
                      0.230035
                                    60 20:34:59
                                                    0.0
 7
         3.97405
                       0.229835
                                    60 20:34:59
                                                    0.0
summary(m)$varcomp
```

```
component std.error z.ratio bound %ch Run -0.004504789 0.002896281 -1.555370 U 0.1 Run:Plant!R 0.229834648 0.042316936 5.431269 P 0.0
```

Now the Run component is negative and the Run:Plant variance estimate is now equal to that for the Residuals Mean Sq for Run:Plant from the anova table.

## Set up an asrtests object

```
current.asrt <- as.asrtests(m)

Calculating denominator DF

print(current.asrt, which = "pseudoanova")</pre>
```

#### Pseudo-anova table for fixed terms

Wald tests for fixed effects. Response: logitP

Df denDF F.inc 1 1550.00 0.0162 (Intercept) 1 59 59.17 0.0000 Host 1 Cadavers 59 37.04 0.0000 2 Ladybird 59 48.26 0.0000 1 59 0.67 0.5158 Host:Cadavers 2 Host:Ladybird 59 0.99 0.3234

```
Cadavers:Ladybird 2 59 3.77 0.0287
Host:Cadavers:Ladybird 2 59 0.44 0.6493
```

The asrtests object contains a wald.tab component which can be printed by specifying that the pseudoanova is printed. The F-values for the fixed terms in this table are the same as those in the anova table.

## Obtain the marginality matrix for the fixed terms

The pstructure function from the dae package (Brien, 2020) produce the marginality matrix for a formula as a side effect and we take advantage of that to obtain the matrix required here.

	Host	Cadavers	Host:Cadavers	Ladybird	Host:Ladybird	Cadavers:Ladybird
Host	1	0	1	0	1	0
Cadavers	0	1	1	0	0	1
Host:Cadavers	0	0	1	0	0	0
Ladybird	0	0	0	1	1	1
Host:Ladybird	0	0	0	0	1	0
Cadavers:Ladybird	0	0	0	0	0	1
Host:Cadavers:Ladybird	0	0	0	0	0	0
Host:Cadavers:Ladybird						

	mobo.caaavorb.baaybrra
Host	1
Cadavers	1
Host:Cadavers	1
Ladybird	1
Host:Ladybird	1
Cadavers:Ladybird	1
<pre>Host:Cadavers:Ladybird</pre>	1

This marginality matrix is interpreted by taking a row term and noting that it is marginal to any column term with a one in this row.

## Choose marginality-compliant model

```
chosen <- chooseModel(current.asrt, terms.marginality = HCL.marg)

Calculating denominator DF
current.asrt <- chosen$asrtests.obj
print(current.asrt, which = "test", omit.columns = c("AIC", "BIC"))</pre>
```

```
#### Sequence of model investigations
```

```
terms DF denDF p action
1 Host:Cadavers:Ladybird 2 59 0.6493 Nonsignificant
```

```
Cadavers:Ladybird 2
                                  59 0.0287
                                                Significant
            Host:Ladybird 1
                                  59 0.3234 Nonsignificant
3
            Host:Cadavers 2
                                  59 0.5158 Nonsignificant
4
                      Host 1
                                                Significant
5
                                  59 0.0000
(chosen$sig.terms)
\lceil \lceil 1 \rceil \rceil
[1] "Cadavers:Ladybird"
[[2]]
[1] "Host"
```

The chooseModel function produces a list with components sig.terms, a list with the terms in the marginality-compliant model, and asrtests.obj, the asrtests object resulting from the model selection. In particular, the asrtests object contains a test.summary that details the tests performed in choosing the model; the AIC and BIC columns are omitted from test.summary because their inclusion has not been requested. Note that chooseModel does not test the main effects for Cadavers or Ladybird, because these are marginal to the significant two-factor interaction Cadavers:Ladybird.

## Form formula for selected model

```
chosen.mod <- paste(unlist(chosen$sig.terms), collapse = " + ")
(chosen.mod <- as.formula(paste("~", chosen.mod)))</pre>
```

~Cadavers:Ladybird + Host

#### Obtain predictions under the chosen model and form an all diffs object

#### Predictions for logitP transform(s) from Host:Ladybird:Cadavers

### Notes:

The original predictions, obtained as described below, have been linearly transformed to form estimated marginal means.

- The predictions are obtained by averaging across the hypertable calculated from model terms constructed solely from factors in the averaging and classify sets.
- Use 'average' to move ignored factors into the averaging set.
- The ignored set: Run

```
Host Ladybird Cadavers predicted.value standard.error upper.halfLeastSignificant.limit
bean - 5 -1.6038338 0.1417454 -1.4080222
```

```
2
                           10
                                   -1.1454308
                                                    0.1417454
                                                                                      -0.9496192
      bean
3
                           20
      bean
                                   -0.7448097
                                                    0.1417454
                                                                                      -0.5489981
4
      bean
                            5
                                   -1.0195475
                                                    0.1417454
                                                                                      -0.8237359
5
                           10
                                   -0.5983440
                                                    0.1417454
                                                                                      -0.4025323
      bean
6
      bean
                           20
                                    0.4786704
                                                    0.1417454
                                                                                       0.6744820
7
  trefoil
                            5
                                   -2.4730339
                                                    0.1417454
                                                                                      -2.2772223
8
   trefoil
                           10
                                   -2.0146309
                                                    0.1417454
                                                                                      -1.8188193
9
  trefoil
                           20
                                   -1.6140098
                                                    0.1417454
                                                                                      -1.4181982
10 trefoil
                            5
                                   -1.8887476
                                                    0.1417454
                                                                                      -1.6929360
11 trefoil
                           10
                                   -1.4675441
                                                    0.1417454
                                                                                      -1.2717325
12 trefoil
                           20
                                   -0.3905297
                                                    0.1417454
                                                                                      -0.1947181
   lower.halfLeastSignificant.limit est.status
                          -1.7996454
                                      Estimable
1
                          -1.3412425
2
                                      Estimable
3
                          -0.9406214
                                      Estimable
4
                          -1.2153592
                                      Estimable
5
                          -0.7941556 Estimable
6
                           0.2828588 Estimable
7
                          -2.6688455 Estimable
8
                          -2.2104426 Estimable
                          -1.8098215 Estimable
9
                          -2.0845593 Estimable
10
                                      Estimable
11
                          -1.6633557
                          -0.5863414 Estimable
12
```

LSD values

```
minimum LSD = 0.3916233 0.3916233
mean LSD = 0.3916233 0.3916233
maximum LSD = 0.3916233 0.3916233
(sed range / mean sed = 7.09e-16 5.67e-16)
```

Setting the terms argument to Host:Ladybird:Cadavers requests predictions for all combinations of the three factors and the linear.transformation argument is used to obtain estimated marginal means (emm) that conform to the chosen model. The wald.tab is supplied so that it can be used to get the degrees of freedom for the t-value to be used in calculating the LSD; the degrees of freedom ot the source for the terms argument will be used. The error.intervals argument has been set to "halfLeast", the meanLSD.type argument to "factor.combination" and the LSDby argument to "Host" so that the average LSD will be calculated for each Host. This necessary because, under the chosen model, the LSDs differ between Hosts. It results in lower.halfLeastSignificant.limit and upper.halfLeastSignificant.limit being added to the predictions component of the alldiffs object.

## Or, caclulate predictions to check first and then transform to conform to chosen model

```
diffs.full <- predictPlus(current.asrt$asreml.obj,</pre>
                           classify = "Host:Ladybird:Cadavers",
                           wald.tab = current.asrt$wald.tab,
                           tables = "none", Vmatrix = TRUE)
```

#### Predictions for logitP transform(s) from Host:Ladybird:Cadavers

## Notes:

The original predictions, obtained as described below, have been linearly transformed to form estimated marginal means.

- The predictions are obtained by averaging across the hypertable calculated from model terms constructed solely from factors in the averaging and classify sets.
- Use 'average' to move ignored factors into the averaging set.
- The ignored set: Run

	Host	Ladybird	${\tt Cadavers}$	<pre>predicted.value</pre>	standard.error	<pre>upper.halfLeastSignificant.limit</pre>
1	bean	-	5	-1.6038338	0.1417454	-1.4080222
2	bean	-	10	-1.1454308	0.1417454	-0.9496192
3	bean	-	20	-0.7448097	0.1417454	-0.5489981
4	bean	+	5	-1.0195475	0.1417454	-0.8237359
5	bean	+	10	-0.5983440	0.1417454	-0.4025323
6	bean	+	20	0.4786704	0.1417454	0.6744820
7	trefoil	-	5	-2.4730339	0.1417454	-2.2772223
8	trefoil	-	10	-2.0146309	0.1417454	-1.8188193
9	trefoil	-	20	-1.6140098	0.1417454	-1.4181982
10	trefoil	+	5	-1.8887476	0.1417454	-1.6929360
11	trefoil	+	10	-1.4675441	0.1417454	-1.2717325
12	trefoil	+	20	-0.3905297	0.1417454	-0.1947181
	lower.ha	alfLeastS:	ignificant	.limit est.statı	ıs	
1			-1.7	996454 Estimab	le	
2			-1.3	3412425 Estimab	le	
3			-0.9	9406214 Estimab	le	
4			-1.2	2153592 Estimab	le	
5			-0.7	941556 Estimab	le	
6			0.2	2828588 Estimab	le	
7			-2.6	688455 Estimab	le	
8			-2.2	2104426 Estimab	le	
9			-1.8	3098215 Estimab	le	
10			-2.0	0845593 Estimab	le	
11			-1.6	633557 Estimab	le	
12			-0.5	863414 Estimab	le	

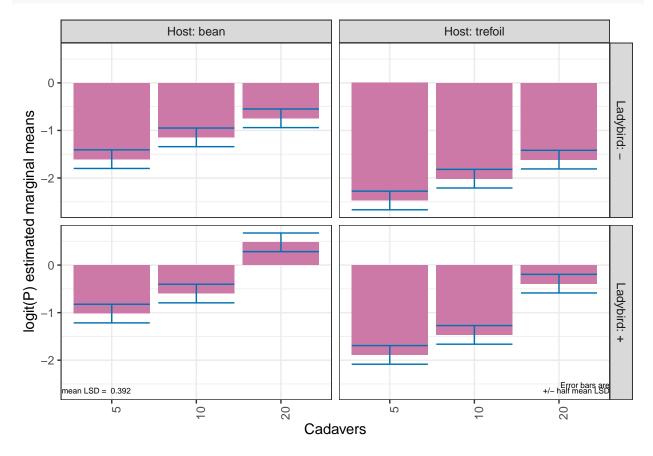
## LSD values

minimum LSD = 0.3916233 0.3916233

mean LSD = 0.3916233 0.3916233

```
maximum LSD = 0.3916233 0.3916233 (sed range / mean sed = 7.09e-16 5.67e-16)
```

## Plot the predictions

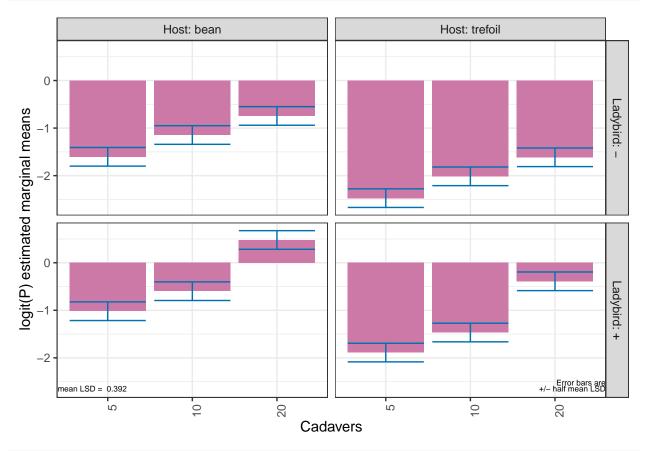


The function plotPredictions uses ggplot to produce the plot and the ggplotFuncs argument allows the addition of ggplot functions to modify the plot. In this case, the facet.grid function is respecified to include prepender functions that modify the labels of the facets to include the factor names. Note the the error bars in the plots are of  $\pm 0.5 LSD$  so that pairs of prediction with nonoverlapping bars are significantly different (Snee, 1981).

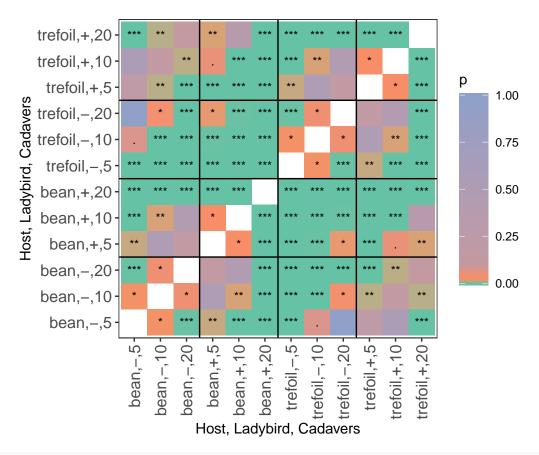
## Get and plot the predictions with a single function call

The predictPresent function combines the functionality of predictPlus and plotPredictions, as demonstrated now. Also, the use of plotPvalues to plot the pairwise p-values is displayed. The predictPresent function has the capability of producing alldiffs objects for multiple terms and these are stored in a list

each of which is named for the term whose alldiffs object it stores. Thus, the term has to be specified in referencing components of diffs.



plotPvalues(diffs\$Host.Ladybird.Cadavers, factors.per.grid = 1, show.sig = TRUE)



## options(width = 90) diffs\$Host.Ladybird.Cadavers\$differences

```
bean,-,5 bean,-,10 bean,-,20
                                                  bean,+,5 bean,+,10 bean,+,20
bean, -,5
              0.00000000 - 0.4584030 - 0.8590241 - 0.5842863 - 1.0054898 - 2.0825042
bean, -, 10
              0.45840297
                          0.0000000 - 0.4006211 - 0.1258833 - 0.5470869 - 1.6241012
bean, -, 20
                                                  0.2747378 -0.1464657 -1.2234801
              0.85902408
                          0.4006211
                                     0.0000000
bean,+,5
              0.58428627
                           0.1258833 -0.2747378
                                                 0.0000000 -0.4212036 -1.4982179
                                                            0.0000000 -1.0770144
bean, +, 10
              1.00548982
                           0.5470869
                                      0.1464657
                                                  0.4212036
bean, +, 20
              2.08250420
                          1.6241012
                                      1.2234801
                                                  1.4982179
                                                             1.0770144
                                                                        0.0000000
trefoil,-,5
             -0.86920012 -1.3276031 -1.7282242 -1.4534864 -1.8746899 -2.9517043
trefoil,-,10 -0.41079715 -0.8692001 -1.2698212 -0.9950834 -1.4162870 -2.4933014
trefoil,-,20 -0.01017604 -0.4685790 -0.8692001 -0.5944623 -1.0156659 -2.0926802
             -0.28491385 -0.7433168 -1.1439379 -0.8692001 -1.2904037 -2.3674180
trefoil,+,5
trefoil,+,10
              0.13628970 -0.3221133 -0.7227344 -0.4479966 -0.8692001 -1.9462145
trefoil,+,20
              1.21330408
                          0.7549011 0.3542800 0.6290178 0.2078143 -0.8692001
             trefoil,-,5 trefoil,-,10 trefoil,-,20 trefoil,+,5 trefoil,+,10 trefoil,+,20
bean, -, 5
               0.8692001
                             0.4107972
                                         0.01017604
                                                       0.2849139
                                                                   -0.1362897
                                                                                 -1.2133041
               1.3276031
                             0.8692001
                                         0.46857901
                                                       0.7433168
                                                                    0.3221133
                                                                                 -0.7549011
bean, -, 10
                                                                                 -0.3542800
                                                                    0.7227344
bean, -, 20
               1.7282242
                             1.2698212
                                         0.86920012
                                                       1.1439379
                                                                                 -0.6290178
bean,+,5
               1.4534864
                             0.9950834
                                         0.59446231
                                                       0.8692001
                                                                    0.4479966
bean, +, 10
               1.8746899
                             1.4162870
                                         1.01566586
                                                       1.2904037
                                                                    0.8692001
                                                                                 -0.2078143
bean,+,20
               2.9517043
                             2.4933014
                                         2.09268024
                                                       2.3674180
                                                                    1.9462145
                                                                                  0.8692001
trefoil,-,5
               0.000000
                            -0.4584030
                                        -0.85902408
                                                      -0.5842863
                                                                   -1.0054898
                                                                                 -2.0825042
trefoil,-,10
               0.4584030
                             0.000000
                                        -0.40062111
                                                      -0.1258833
                                                                   -0.5470869
                                                                                 -1.6241012
trefoil,-,20
                                         0.00000000
                                                       0.2747378
               0.8590241
                             0.4006211
                                                                   -0.1464657
                                                                                 -1.2234801
```

```
trefoil,+,5
               0.5842863
                            0.1258833
                                       -0.27473781
                                                      0.0000000
                                                                  -0.4212036
                                                                               -1.4982179
                                                                   0.0000000
trefoil,+,10
               1.0054898
                            0.5470869
                                        0.14646574
                                                      0.4212036
                                                                               -1.0770144
trefoil,+,20
               2.0825042
                            1.6241012
                                        1.22348012
                                                      1.4982179
                                                                   1.0770144
                                                                                0.000000
options(width = 90)
print(diffs$Host.Ladybird.Cadavers$sed)
              bean,-,5 bean,-,10 bean,-,20 bean,+,5 bean,+,10 bean,+,20 trefoil,-,5
bean, -, 5
                    NA 0.1957142 0.1957142 0.1957142 0.1957142 0.1957142
                                                                            0.1129957
bean, -, 10
                              NA 0.1957142 0.1957142 0.1957142 0.1957142
                                                                            0.2259913
             0.1957142
bean, -, 20
             0.1957142 0.1957142
                                        NA 0.1957142 0.1957142 0.1957142
                                                                            0.2259913
bean,+,5
             0.1957142 0.1957142 0.1957142
                                                  NA 0.1957142 0.1957142
                                                                            0.2259913
bean,+,10
             0.1957142 0.1957142 0.1957142 0.1957142
                                                                            0.2259913
             0.1957142 \ 0.1957142 \ 0.1957142 \ 0.1957142 \ 0.1957142
                                                                            0.2259913
bean,+,20
trefoil,-,5 0.1129957 0.2259913 0.2259913 0.2259913 0.2259913 0.2259913
                                                                                   NA
trefoil,-,10 0.2259913 0.1129957 0.2259913 0.2259913 0.2259913 0.2259913
                                                                            0.1957142
trefoil,-,20 0.2259913 0.2259913 0.1129957 0.2259913 0.2259913 0.2259913
                                                                            0.1957142
trefoil,+,5 0.2259913 0.2259913 0.1129957 0.2259913 0.2259913
                                                                            0.1957142
trefoil,+,10 0.2259913 0.2259913 0.2259913 0.1129957 0.2259913
                                                                            0.1957142
trefoil,+,20 0.2259913 0.2259913 0.2259913 0.2259913 0.2259913 0.1129957
                                                                            0.1957142
             trefoil,-,10 trefoil,-,20 trefoil,+,5 trefoil,+,10 trefoil,+,20
bean, -, 5
                0.2259913
                             0.2259913
                                         0.2259913
                                                      0.2259913
                                                                    0.2259913
bean,-,10
                0.1129957
                             0.2259913
                                         0.2259913
                                                      0.2259913
                                                                    0.2259913
bean,-,20
                             0.1129957
                                                                    0.2259913
                0.2259913
                                         0.2259913
                                                      0.2259913
bean,+,5
                0.2259913
                             0.2259913
                                         0.1129957
                                                      0.2259913
                                                                    0.2259913
bean,+,10
                0.2259913
                             0.2259913
                                         0.2259913
                                                      0.1129957
                                                                    0.2259913
                0.2259913
                             0.2259913
                                         0.2259913
                                                      0.2259913
                                                                    0.1129957
bean,+,20
trefoil,-,5
                0.1957142
                             0.1957142
                                         0.1957142
                                                      0.1957142
                                                                    0.1957142
trefoil,-,10
                       NA
                             0.1957142
                                         0.1957142
                                                      0.1957142
                                                                    0.1957142
trefoil,-,20
                0.1957142
                                         0.1957142
                                                      0.1957142
                                                                    0.1957142
                0.1957142
                             0.1957142
                                                      0.1957142
trefoil,+,5
                                                NA
                                                                    0.1957142
trefoil,+,10
                0.1957142
                             0.1957142
                                         0.1957142
                                                                    0.1957142
                                                             NA
trefoil,+,20
                0.1957142
                             0.1957142
                                         0.1957142
                                                      0.1957142
                                                                           NA
```

## Perform the analysis with just selected model fitted

The model with nonsignificant fixed terms dropped is obtained in order to compare it with the fit when they are retained and the etimated marginal means for the chosen model are obtained.

#### Pseudo-anova table for fixed terms

Wald tests for fixed effects.

Response: logitP

```
Df denDF
                            F.inc
                                      Pr
(Intercept)
                  1
                      1 1550.00 0.0162
Host
                       64
                            60.88 0.0000
                  1
Cadavers
                  2
                       64
                            38.12 0.0000
                       64 49.65 0.0000
Ladybird
                  1
Cadavers:Ladybird 2
                       64
                             3.88 0.0256
diffs.red <- predictPlus(red.asrt$asreml.obj,</pre>
                        classify = "Host:Ladybird:Cadavers",
                        wald.tab = current.asrt$wald.tab,
                        error.intervals = "halfLeast",
                        meanLSD.type = "factor.combination", LSDby = "Host",
                        tables = "predictions")
```

#### Predictions for logitP from Host:Ladybird:Cadavers

#### Notes:

- The ignored set: Run

- The predictions are obtained by averaging across the hypertable calculated from model terms constructed solely from factors in the averaging and classify sets.
- Use 'average' to move ignored factors into the averaging set.

```
Host Ladybird Cadavers predicted.value standard.error
     bean
                                 -1.6038338
1
                         5
                                                 0.1398332
2
     bean
                         10
                                 -1.1454308
                                                 0.1398332
3
                         20
     bean
                                 -0.7448097
                                                 0.1398332
4
     bean
                         5
                                 -1.0195475
                                                 0.1398332
5
                        10
                                 -0.5983440
                                                 0.1398332
     bean
6
     bean
                         20
                                 0.4786704
                                                 0.1398332
7 trefoil
                        5
                                 -2.4730339
                                                 0.1398332
8 trefoil
                        10
                                 -2.0146309
                                                 0.1398332
9 trefoil
                         20
                                 -1.6140098
                                                 0.1398332
10 trefoil
                         5
                                 -1.8887476
                                                 0.1398332
11 trefoil
                         10
                                 -1.4675441
                                                 0.1398332
12 trefoil
                         20
                                 -0.3905297
                                                 0.1398332
```

upper.halfLeastSignificant.limit lower.halfLeastSignificant.limit est.status -1.7968734 Estimable -1.4107942 1 2 -0.9523913 -1.3384704 Estimable 3 -0.5517702 -0.9378493 Estimable -1.2125871 Estimable 4 -0.8265080 -0.4053044 5 -0.7913835 Estimable 6 0.6717100 0.2856308 Estimable -2.2799944 7 -2.6660735 Estimable 8 -1.8215914 -2.2076705 Estimable 9 -1.4209703 -1.8070494 Estimable -2.0817872 Estimable 10 -1.6957081

```
11
                         -1.2745045
                                                           -1.6605837
                                                                       Estimable
12
                         -0.1974902
                                                           -0.5835693
                                                                       Estimable
LSD values
minimum LSD = 0.3860791 0.3860791
mean LSD = 0.3860791 0.3860791
maximum LSD = 0.3860791 0.3860791
(sed range / mean sed = 2.44e-15 2.3e-15)
options(width = 90)
print(diffs.red$sed)
              bean,-,5 bean,-,10 bean,-,20 bean,+,5 bean,+,10 bean,+,20 trefoil,-,5
bean,-,5
                    NA 0.1929435 0.1929435 0.1929435 0.1929435 0.1929435
                                                                             0.1113960
bean,-,10
                              NA 0.1929435 0.1929435 0.1929435 0.1929435
                                                                             0.2227920
             0.1929435
bean, -, 20
             0.1929435 0.1929435
                                        NA 0.1929435 0.1929435 0.1929435
                                                                             0.2227920
bean,+,5
             0.1929435 0.1929435 0.1929435
                                                   NA 0.1929435 0.1929435
                                                                            0.2227920
bean,+,10
             0.1929435 0.1929435 0.1929435 0.1929435
                                                             NA 0.1929435
                                                                            0.2227920
             0.1929435 0.1929435 0.1929435 0.1929435 0.1929435
                                                                            0.2227920
bean,+,20
                                                                        NA
trefoil,-,5 0.1113960 0.2227920 0.2227920 0.2227920 0.2227920 0.2227920
                                                                                    NΑ
trefoil,-,10 0.2227920 0.1113960 0.2227920 0.2227920 0.2227920 0.2227920
                                                                             0.1929435
trefoil,-,20 0.2227920 0.2227920 0.1113960 0.2227920 0.2227920 0.2227920
                                                                            0.1929435
trefoil,+,5 0.2227920 0.2227920 0.1113960 0.2227920 0.2227920
                                                                             0.1929435
trefoil,+,10 0.2227920 0.2227920 0.2227920 0.2227920 0.1113960 0.2227920
                                                                             0.1929435
trefoil,+,20 0.2227920 0.2227920 0.2227920 0.2227920 0.2227920 0.1113960
                                                                             0.1929435
             trefoil,-,10 trefoil,-,20 trefoil,+,5 trefoil,+,10 trefoil,+,20
bean, -,5
                0.2227920
                             0.2227920
                                          0.2227920
                                                       0.2227920
                                                                    0.2227920
                             0.2227920
                                          0.2227920
                                                       0.2227920
bean, -, 10
                0.1113960
                                                                    0.2227920
bean,-,20
                0.2227920
                             0.1113960
                                         0.2227920
                                                       0.2227920
                                                                    0.2227920
bean,+,5
                0.2227920
                             0.2227920
                                         0.1113960
                                                       0.2227920
                                                                    0.2227920
                             0.2227920
                                         0.2227920
                                                       0.1113960
bean,+,10
                0.2227920
                                                                    0.2227920
bean, +, 20
                0.2227920
                             0.2227920
                                         0.2227920
                                                       0.2227920
                                                                    0.1113960
trefoil,-,5
                0.1929435
                             0.1929435
                                         0.1929435
                                                       0.1929435
                                                                    0.1929435
trefoil,-,10
                             0.1929435
                                         0.1929435
                                                       0.1929435
                                                                    0.1929435
                       NA
trefoil,-,20
                0.1929435
                                    NA
                                         0.1929435
                                                       0.1929435
                                                                    0.1929435
trefoil,+,5
                0.1929435
                             0.1929435
                                                 NA
                                                       0.1929435
                                                                    0.1929435
trefoil,+,10
                0.1929435
                             0.1929435
                                         0.1929435
                                                              NA
                                                                    0.1929435
```

## References

trefoil,+,20

0.1929435

0.1929435

Brien, C. J. (2020a) asremlPlus: Augments ASReml-R in fitting mixed models and packages generally in exploring prediction differences. Version 4.1-37. http://cran.r-project.org/package=asremlPlus/ or http://chris.brien.name/rpackages/.

0.1929435

0.1929435

NA

Brien, C. J. (2020b) dae: Functions useful in the design and ANOVA of experiments. Version 3.1-22. http://cran.r-project.org/package=dae/ or http://chris.brien.name/rpackages/.

Butler, D. G., Cullis, B. R., Gilmour, A. R., Gogel, B. J., & Thompson, R. (2018). ASReml-R reference

manual. Version 4. Retrieved from http://asreml.org.

Littell, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R. D., & Schabenberger, O. (2006). SAS for Mixed Model. (2nd ed.). Cary, N.C.: SAS Press.

R Core Team (2020) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.r-project.org.

Snee, R. D. (1981). Graphical Display and Assessment of Means. Biometrics, 37, 835–836.

Welham, S. J., Gezan, S. A., Clark, S. J., & Mead, A. (2014). Statistical Methods in Biology: Design and Analysis of Experiments and Regression. Boca Raton: Chapman and Hall/CRC.