# Using lm with asremlPlus for the Ladybird example from Welham et al. (2014)

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# Introduction

This vignette shows how to use asremlPlus (Brien, 2025a), and dae (Brien, 2024b), for exploring and presenting predictions (estimated marginal means: EMMs) from a linear mixed model analysis, the predictions having been produced using lmerTest (Kuznetsova et al., 2017), lm and emmeans (Lenth, 2023). Here, asremlPlus, dae, lmerTest and emmeans are packages for the R Statistical Computing environment (R Core Team, 2025) and lm is available from stats and is included in R.

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 (-, +). These are randomized to the containers within a run so that each is replicated 3 times within a run. The response 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(lmerTest))
packageVersion("lmerTest")

## [1] '3.1.3'
suppressMessages(library(emmeans))

## Warning: package 'emmeans' was built under R version 4.5.1

packageVersion("emmeans")

## [1] '1.11.2'
suppressMessages(library(asremlPlus))
packageVersion("asremlPlus")
```

```
## [1] '4.4.51'
```

```
suppressMessages(library(dae))
packageVersion("dae")

## [1] '3.2.31'

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

	Df	Sum Sq	Mean Sq	F 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
${\tt Host:Cadavers:Ladybird}$	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 lmerTest and lm to analyse the logits

# Mixed model analysis of logits

```
m1.lmer <- lmerTest::lmer(logitP ~ Host*Cadavers*Ladybird + (1 Run),
                     data=Ladybird.dat)
boundary (singular) fit: see help('isSingular')
summary(m1.lmer)
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: logitP ~ Host * Cadavers * Ladybird + (1 | Run)
  Data: Ladybird.dat
REML criterion at convergence: 102.8
Scaled residuals:
   Min
          1Q Median
                       3Q
-1.9633 -0.5217 0.1360 0.5789 2.1896
Random effects:
                Variance Std.Dev.
Groups Name
      (Intercept) 0.0000 0.0000
Run
Residual
                 0.2271
                         0.4766
Number of obs: 72, groups: Run, 2
Fixed effects:
                           Estimate Std. Error
                                                 df t value Pr(>|t|)
                          -1.603097 0.194560 60.000000 -8.240 1.91e-11
(Intercept)
Hosttrefoil
                          Cadavers10
                           0.919229 0.275149 60.000000 3.341 0.00144
Cadavers20
Ladybird+
                           0.547710  0.275149  60.000000  1.991  0.05109
Hosttrefoil:Cadavers10
                          Hosttrefoil:Cadavers20
                         Hosttrefoil:Ladybird+
Cadavers10:Ladybird+
                          Cadavers20:Ladybird+
                           Hosttrefoil:Cadavers10:Ladybird+ 0.005698 0.550299 60.000000 0.010 0.99177
Hosttrefoil:Cadavers20:Ladybird+ 0.449979 0.550299 60.000000 0.818 0.41676
Correlation of Fixed Effects:
         (Intr) Hsttrf Cdvr10 Cdvr20 Ldybr+ Hs:C10 Hs:C20 Hst:L+ C10:L+ C20:L+ H:C10:
Hosttrefoil -0.707
Cadavers10 -0.707 0.500
Cadavers20 -0.707 0.500 0.500
Ladybird+ -0.707 0.500 0.500 0.500
Hsttrfl:C10 0.500 -0.707 -0.707 -0.354 -0.354
Hsttrf1:C20 0.500 -0.707 -0.354 -0.707 -0.354 0.500
Hsttrf1:Ld+ 0.500 -0.707 -0.354 -0.354 -0.707 0.500 0.500
Cdvrs10:Ld+ 0.500 -0.354 -0.707 -0.354 -0.707 0.500 0.250 0.500
Cdvrs20:Ld+ 0.500 -0.354 -0.354 -0.707 -0.707 0.250 0.500 0.500 0.500
Hstt:C10:L+ -0.354 0.500 0.500 0.250 0.500 -0.707 -0.354 -0.707 -0.707 -0.354
Hstt:C20:L+ -0.354  0.500  0.250  0.500  -0.354  -0.707  -0.707  -0.354  -0.707  0.500
optimizer (nloptwrap) convergence code: 0 (OK)
```

```
boundary (singular) fit: see help('isSingular')
```

As expected the Run component is bound at zero, leading to a singular model. This results in a change in the estimate of the residual variance to 0.227. To allow for a negative estimate we will redo the analysis with Run fixed, because with lme4 (lmerTest) one cannot unconstrain the Run component to allow it to be negative. 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.

# Analyse with Reps fixed using 1m to make the analysis equivalent to ANOVA

The function 1m has to be used because there are no random terms; 1me4 cannot be used because it requires at least one random term.

Analysis of Variance Table

```
Response: logitP
```

	Df	Sum Sq	${\tt Mean \ Sq}$	${\tt F} \ {\tt value}$	Pr(>F)
Run	1	0.0677	0.0677	0.2944	0.58946
Host	1	13.5992	13.5992	59.1720	1.815e-10
Cadavers	2	17.0274	8.5137	37.0444	3.784e-11
Ladybird	1	11.0907	11.0907	48.2571	3.329e-09
Host:Cadavers	2	0.3078	0.1539	0.6695	0.51579
Host:Ladybird	1	0.2279	0.2279	0.9916	0.32341
Cadavers:Ladybird	2	1.7349	0.8675	3.7744	0.02867
${\tt Host:Cadavers:Ladybird}$	2	0.1999	0.1000	0.4350	0.64932
Residuals	59	13.5596	0.2298		

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

#### Obtain the marginality matrix for the fixed terms

The pstructure function from the dae package (Brien, 2024b) 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	<pre>Host:Ladybird</pre>	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:Cad	lavers:Lad	ybird				
Host			1				
Cadavers			1				
Host:Cadavers			1				
Ladybird			1				
Host:Ladybird			1				
Cadavers:Ladybird			1				
Host:Cadavers:Ladybird			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

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

```
terms DF denDF
                                                 action
1 Host:Cadavers:Ladybird 2
                               59 0.6493 Nonsignificant
      Cadavers:Ladybird 2
                                            Significant
2
                               59 0.0287
3
           Host:Ladybird 1
                               59 0.3234 Nonsignificant
4
           Host:Cadavers 2
                               59 0.5158 Nonsignificant
5
                    Host 1
                               59 0.0000
                                            Significant
```

```
(chosen\sig.terms)
```

```
[[1]]
[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 choose.summary, a data.frame that details the tests performed in choosing the model. 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

## Form predictions that conform to the chosen model

Use emmeans to get the predictions and associated statistics for the full model.

```
HCL.emm <- emmeans::emmeans(m1.lmer, specs = ~ Host:Cadavers:Ladybird)
HCL.preds <- summary(HCL.emm)
den.df <- min(HCL.preds$df)
HCL.vcov <- vcov(HCL.emm)</pre>
```

Setting the specs argument to Host:Ladybird:Cadavers requests predictions for all combinations of the three factors.

#### Modify HCL.preds to be compatible with a predictions.frame

Basically, this is an exercise in renaming the columns in the data.frame containing the predictions.

```
names(HCL.preds)
[1] "Host"
               "Cadavers" "Ladybird" "emmean"
                                                  "SE"
                                                              "df"
                                                                         "lower.CL" "upper.CL"
HCL.preds <- as.predictions.frame(HCL.preds, predictions = "emmean",</pre>
                                   se = "SE", interval.type = "CI",
                                   interval.names = c("lower.CL", "upper.CL"))
names(HCL.preds)
[1] "Host"
                              "Cadavers"
                                                        "Ladybird"
[4] "predicted.value"
                              "standard.error"
[7] "lower.Confidence.limit" "upper.Confidence.limit" "est.status"
```

Form an alldiffs object with predictions obtained with emmeans

The functions allDifferences is used to form the alldiffs.obj that contains a predictions component, along with components related to pairwise comparisons. The predictions component contains upper and lower confidence limits produced by emmeans. The tdf is supplied so that it can be used to get the degrees of freedom for the t-value to be used in calculating the error intervals.

#### Transform the prediction to conform to chosen model

The linTransform function is used to obtain estimated marginal means (EMMs) that conform to the chosen model. Because we would prefer error intervals based on  $\pm 0.5 LSD$ , the error intervals argument has been set to "halfLeast", the LSDtype 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 replacing the limits based on the confidence intervals in the predictions component of the resulting alldiffs object.

```
Joining with 'by = join_by(fac.comb)'
Joining with 'by = join_by(Host)'
```

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

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

```
Host Ladybird Cadavers predicted.value standard.error
      bean
                           5
                                  -1.6038338
                                                  0.1485977 47.2
1
2
     bean
                          10
                                  -1.1454308
                                                  0.1485977 47.2
3
     bean
                          20
                                  -0.7448097
                                                  0.1485977 47.2
4
     bean
                          5
                                  -1.0195475
                                                  0.1485977 47.2
5
                          10
                                  -0.5983440
                                                  0.1485977 47.2
     bean
6
     bean
                          20
                                   0.4786704
                                                  0.1485977 47.2
7 trefoil
                          5
                                                  0.1485977 47.2
                                  -2.4730339
  trefoil
                          10
                                  -2.0146309
                                                  0.1485977 47.2
9 trefoil
                          20
                                                  0.1485977 47.2
                                  -1.6140098
10 trefoil
                          5
                                  -1.8887476
                                                  0.1485977 47.2
11 trefoil
                          10
                                  -1.4675441
                                                  0.1485977 47.2
12 trefoil
                          20
                                  -0.3905297
                                                  0.1485977 47.2
```

```
upper.halfLeastSignificant.limit lower.halfLeastSignificant.limit est.status
1
                         -1.4081535
                                                           -1.7995140 Estimable
2
                                                           -1.3411111 Estimable
                         -0.9497506
3
                         -0.5491295
                                                           -0.9404900 Estimable
4
                                                           -1.2152278 Estimable
                         -0.8238673
5
                         -0.4026637
                                                           -0.7940242 Estimable
6
                          0.6743507
                                                           0.2829901 Estimable
7
                                                           -2.6687142 Estimable
                         -2.2773537
8
                         -1.8189507
                                                          -2.2103112 Estimable
9
                         -1.4183296
                                                          -1.8096901 Estimable
10
                         -1.6930674
                                                           -2.0844279 Estimable
                                                          -1.6632243 Estimable
11
                         -1.2718638
12
                         -0.1948495
                                                          -0.5862100 Estimable
```

LSD values

```
minimum LSD = 0.3913605 0.3913605

mean LSD = 0.3913605 0.3913605

maximum LSD = 0.3913605 0.3913605

(sed range / mean sed = 2.45e-14 2.41e-14 )
```

The above LSD values can only be used to compare pairs of EMMs for the same Host.

#### Explore the LSDs

To investigate the errors that would result from using the overall LSDs as opposed to the LSDs computed for each Host, the exploreLSDs, pickLSDstatistics and plotLSDerrors functions are used, firstly with the default value of "overall" for LSDtype and finally with the LSDtype set to "factor.combination" and LSDby to "Host".

The exploreLSDs function produces tables of statistics for the LSDs computed for the settings of the LSDtype and LSDby; the settings of these arguments does not have to match those used in producing the alldiffs object. For LSDtype set to "overall', a single LSD statistic is computed that is the based on the standard errors of all pairwise differences. To ascertain the errors that arise from using this LSD value for determining, for all pairwise comparisons, whether a comparison is significant, exploreLSDs compares the results using the LSD value with the p-values in the p.differences component of the alldiffs object. For LSDtype set to "factor.combination" and LSDby to "Host", the LSD statistics are calculated from standard errors of the pairwise differences for each Host. Examination of the sed component of diffs reveals that there are only three different values for the standard errors of pairwise differences and, hence, only three unique values for the LSD. Of the statistics shown, there is no single value that will not result in errors.

Here are the tables of the numbers of false positive and negative error in using the values of the various LSD statistics for determining the significance of the 66 pairwise comparisons of the 12 predictions.

```
exploreLSDs(diffs, LSDtype = "overall")
```

```
Statistics calculated from LSD values
####
           min
                 quant10
                           quant25
                                        mean
                                                median
                                                          quant75
                                                                    quant90
1 66 0.2259521 0.3913605 0.3913605 0.4087627 0.3913605 0.4519042 0.4519042 0.4519042
     False positives resulting from the use of various LSD statistics
           c min quant10 quant25 mean median quant75 quant90 max
false.pos 66
                       2
                               2
                                    2
                                            2
                                                    0
#### False negatives resulting from the use of various LSD statistics
           c min quant10 quant25 mean median quant75 quant90 max
                                    2
                                                    4
false.neg 66
               0
                       Λ
                               0
                                           0
                                                            4
```

The function findLSDminerrors can be used to find a value of the LSD that minimizes the number of false positive (negative) errors i.e. declaring a pairwise difference to be significant (not significant) when it is not significant (significant). The function has an argument false.pos.wt that specifies how many false negatives are equivalent to one false positive; the default for false.pos.wt is 10, which is a conservative approach because it greatly favours false negatives over false positives.

#### (findLSDminerrors(diffs))

#### \$overall

#### \$overall\$lsd

- [1] 0.3913605 0.3913605 0.3913605 0.3913605 0.3913605 0.3913605 0.3913605 0.3913605
- [10] 0.3913605 0.3913605 0.3913605 0.3913605 0.3913605 0.3913605 0.2259521 0.4519042 0.4519042
- $[19] \ \ 0.4519042 \ \ 0.4519042 \ \ 0.4519042 \ \ 0.4519042 \ \ 0.2259521 \ \ 0.4519042 \ \ 0.45$
- $[28] \quad 0.3913605 \quad 0.4519042 \quad 0.4519042 \quad 0.2259521 \quad 0.4519042 \quad 0.4519042 \quad 0.4519042 \quad 0.3913605 \quad 0.3913605$
- $[37] \quad 0.4519042 \quad 0.4519042 \quad 0.4519042 \quad 0.2259521 \quad 0.4519042 \quad 0.4519042 \quad 0.3913605 \quad 0.3913605 \quad 0.3913605$
- $[46] \quad 0.4519042 \quad 0.4519042 \quad 0.4519042 \quad 0.4519042 \quad 0.2259521 \quad 0.4519042 \quad 0.3913605 \quad 0.3913605$
- $[55] \ \ 0.3913605 \ \ 0.4519042 \ \ 0.4519042 \ \ 0.4519042 \ \ 0.4519042 \ \ 0.259521 \ \ 0.3913605 \ \ 0.3913605$
- [64] 0.3913605 0.3913605 0.3913605

#### \$overall\$dif

- $\begin{bmatrix} 1 \end{bmatrix} \ -0.45840297 \ -0.85902408 \ -0.40062111 \ -0.58428627 \ -0.12588330 \ \ 0.27473781 \ -1.00548982$
- [8] -0.54708685 -0.14646574 -0.42120355 -2.08250420 -1.62410123 -1.22348012 -1.49821793
- [15] -1.07701438 0.86920012 1.32760308 1.72822420 1.45348638 1.87468994 2.95170432
- $\begin{bmatrix} 22 \end{bmatrix} \quad 0.41079715 \quad 0.86920012 \quad 1.26982123 \quad 0.99508342 \quad 1.41628697 \quad 2.49330135 \quad -0.45840297 \quad 1.41628697 \quad 2.49330135 \quad -0.45840297 \quad 1.41628697 \quad 1.4166867 \quad 1.416867 \quad 1.4166867 \quad 1.4166867 \quad 1.4166867 \quad 1.4166867 \quad 1.4166867 \quad$
- [29] 0.01017604 0.46857901 0.86920012 0.59446231 1.01566586 2.09268024 -0.85902408
- [36] -0.40062111 0.28491385 0.74331682 1.14393793 0.86920012 1.29040367 2.36741805
- $[43] \ -0.58428627 \ -0.12588330 \ \ 0.27473781 \ -0.13628970 \ \ 0.32211327 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.27473781 \ \ 0.13628970 \ \ 0.32211327 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.13628970 \ \ 0.32211327 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.32211327 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.32211327 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.32211327 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.32211327 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.32211327 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.32211327 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.32211327 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.32211327 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.32211327 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.32211327 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.32211327 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.72273438 \ \ 0.44799657 \ \ 0.72273438 \ \ 0.72273434 \ \ 0.72273434 \ \ 0.72273434 \ \ 0.72273434 \ \ 0.72273438 \ \ 0.72273434 \ \ 0.72273434 \ \ 0.72273434 \ \ 0.72273434 \$
- $\begin{bmatrix} 50 \end{bmatrix} \quad 0.86920012 \quad 1.94621450 \quad -1.00548982 \quad -0.54708685 \quad -0.14646574 \quad -0.42120355 \quad -1.21330408$
- [57] -0.75490112 -0.35428000 -0.62901781 -0.20781426 0.86920012 -2.08250420 -1.62410123
- [64] -1.22348012 -1.49821793 -1.07701438

#### \$overall\$sig.actual

- [1] TRUE TRUE TRUE TRUE FALSE FALSE TRUE TRUE FALSE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
- [31] TRUE TRUE TRUE TRUE TRUE TRUE FALSE TRUE TRUE TRUE TRUE TRUE TRUE FALSE FALSE [46] FALSE FALSE TRUE TRUE TRUE TRUE FALSE TRUE TRUE TRUE FALSE TRUE FALSE TRUE FALSE
- [61] TRUE TRUE TRUE TRUE TRUE TRUE

LSD false.pos false.neg false.criterion

overall 0.4486447 0 4

#### (minLSDpos <- findLSDminerrors(diffs, false.pos.wt = 1))</pre>

#### \$overall

#### \$overall\$lsd

- $[1] \quad 0.3913605 \quad 0.3913605$
- [10] 0.3913605 0.3913605 0.3913605 0.3913605 0.3913605 0.3913605 0.2259521 0.4519042 0.4519042
- [19] 0.4519042 0.4519042 0.4519042 0.4519042 0.2259521 0.4519042 0.4519042 0.4519042 0.4519042
- [28] 0.3913605 0.4519042 0.4519042 0.2259521 0.4519042 0.4519042 0.4519042 0.3913605 0.3913605
- [37] 0.4519042 0.4519042 0.4519042 0.2259521 0.4519042 0.4519042 0.3913605 0.3913605
- [46] 0.4519042 0.4519042 0.4519042 0.4519042 0.2259521 0.4519042 0.3913605 0.3913605
- $[55] \ \ 0.3913605 \ \ 0.4519042 \ \ 0.4519042 \ \ 0.4519042 \ \ 0.4519042 \ \ 0.2259521 \ \ 0.3913605 \ \ 0.3913605$
- [64] 0.3913605 0.3913605 0.3913605

#### \$overall\$dif

```
 \begin{bmatrix} 1 \end{bmatrix} -0.45840297 -0.85902408 -0.40062111 -0.58428627 -0.12588330 \quad 0.27473781 -1.00548982 
 \begin{bmatrix} 8 \end{bmatrix} \ -0.54708685 \ -0.14646574 \ -0.42120355 \ -2.08250420 \ -1.62410123 \ -1.22348012 \ -1.49821793 
[15] -1.07701438
                   0.86920012
                                 1.32760308
                                              1.72822420
                                                            1.45348638
                                                                         1.87468994
     0.41079715
                   0.86920012
                                 1.26982123
                                              0.99508342
                                                           1.41628697
[22]
                                                                         2.49330135 -0.45840297
      0.01017604
                   0.46857901
                                 0.86920012
                                              0.59446231
                                                           1.01566586
                                                                         2.09268024 -0.85902408
[36] -0.40062111
                   0.28491385
                                 0.74331682
                                              1.14393793
                                                           0.86920012
                                                                         1.29040367
                                                                                      2.36741805
[43] -0.58428627 -0.12588330
                                0.27473781 -0.13628970
                                                            0.32211327
                                                                         0.72273438
                                                                                      0.44799657
[50]
     0.86920012
                   1.94621450 -1.00548982 -0.54708685 -0.14646574 -0.42120355 -1.21330408
[57] -0.75490112 -0.35428000 -0.62901781 -0.20781426
                                                           0.86920012 -2.08250420 -1.62410123
[64] -1.22348012 -1.49821793 -1.07701438
```

#### \$overall\$sig.actual

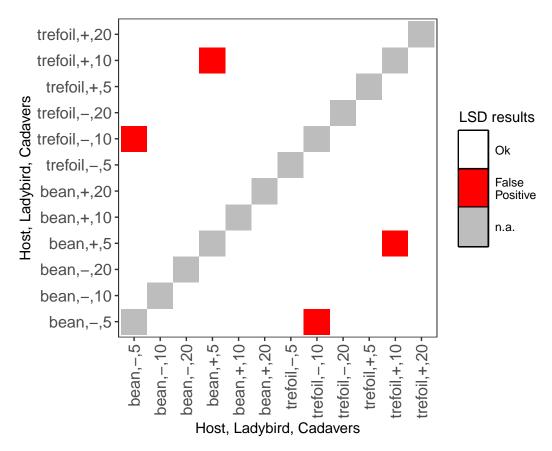
```
TRUE
            TRUE
                         TRUE FALSE FALSE
                                                                TRUE
                                                                       TRUE
                                                                              TRUE
                                                                                    TRUE
                                                                                          TRUE
[1]
                   TRUE
                                            TRUE
                                                   TRUE FALSE
                                                                                                 TRUE
[16]
      TRUE
            TRUE
                   TRUE
                         TRUE
                                TRUE
                                      TRUE FALSE
                                                   TRUE
                                                          TRUE
                                                                 TRUE
                                                                       TRUE
                                                                              TRUE
                                                                                    TRUE FALSE
                                                                                                 TRUE
[31]
      TRUE
                                      TRUE FALSE
                                                                TRUE
                                                                       TRUE
                                                                                    TRUE FALSE FALSE
            TRUE
                   TRUE
                         TRUE
                                TRUE
                                                   TRUE
                                                          TRUE
                                                                             TRUE
[46] FALSE FALSE
                   TRUE FALSE
                                TRUE
                                      TRUE
                                             TRUE
                                                   TRUE FALSE
                                                                TRUE
                                                                       TRUE
                                                                             TRUE FALSE
                                                                                          TRUE FALSE
                   TRUE
[61]
      TRUE
            TRUE
                         TRUE
                                TRUE
                                      TRUE
```

```
LSD false.pos false.neg false.criterion overall 0.3547448 2 0 2
```

The smallest value of the LSD that results in the minimum weighted sum of the false positives and negatives is 0.4489466 when the weight is 10 and is 0.354983 when the weight is one. These values result in 4 false negatives and 2 false positives respectively.

To see which of the two pairwise comparisons will be falsely identified as being significant when the LSD value is set to minimum LSD, the LSDs stored in the alldiffs object need to be recalculated to be based on this LSD value. Then plot the errors or save the return values obtained using the function plotLSDerrors. The plot below shows that the two pairs whose differences are incorrectly identified as significant have the same level of the Ladybird factor, but differ in both of the levels for the Host and Cadaver factors.

```
diffs.overall <- recalcLSD(diffs, LSDtype = "supplied", LSDsupplied = minLSDpos["LSD"])
plotLSDerrors(diffs.overall)</pre>
```



This raises the question of whether the minimum LSD should be used. There are at least four alternatives: (i) use it without restriction, on the basis that it can be concluded that using it is unlikely to result in seriously flawed conclusions; (ii) use it with the restriction that it only be applied to assess pairwise comparisons that have the same Host or the same Cadaver treatment; (i11) investigate the use of the minimum LSD with only false negative errors; and (iv) rather than use an overall LSD value, use LSD values computed from the LSDs within each Host level.

Because LSDtype was set to "factor.combination" and LSDby to "Host" in forming the object diffs, the LSDs for alternative (iv) are stored in the LSD component of the object diffs. Printing out the LSD component will summarize how those LSD values perform. Otherwise, the following call to exploreLSDs will display the properties of the LSDs for various LSD statistics:

```
exploreLSDs(diffs, LSDtype = "factor.combination", LSDby = "Host")
```

The following shows the contents of the LSD component of diffs:

trefoil 15 0.3913605 0.3913605 0.3913605

```
(diffs$LSD)

c minLSD meanLSD maxLSD assignedLSD accuracyLSD falsePos falseNeg
bean 15 0.3913605 0.3913605 0.3913605 0.3913605 1.886492e-14 0 0
```

0.3913605 1.843939e-14

0

0

Because the minimum and maximum LSD values are equal, it follows that there is only one value of the LSD for all pairwise comparisons within each Host level and it happens that the values for the two Hosts are also equal. The table shows that zero false positive and negative errors will result from the use of the value of 0.39 for the 30 within-Host comparisons. Thus, using findLSDminerrors(diffs, LSDtype = "factor.combinations", LSDby = "Host") to select the minimum LSDs would only confirm what is already obvious.

#### Plot the predictions

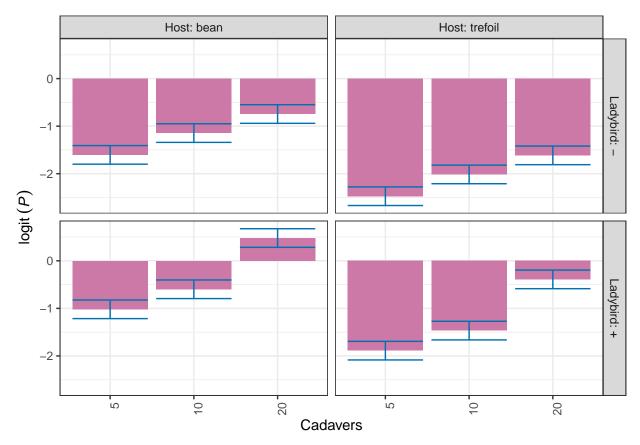


Figure 1. Estimated marginal means (EMMs) for logit(P), where P is the proportion of live aphids that were infected, for two Hosts, two Ladybird levels and three Cadaver levels. Error bars are an EMM  $\pm$  half-LSD (5%). The two EMMs for the same Host are significantly different ( $p \le 0.05$ ) if their error bars do not overlap.

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 statement in the legend of Figure 1 that restricts the use of the error bars to determining the significance of differences for the pairwise comparisons of EMMs for the same Host.

```
plotPvalues(diffs, factors.per.grid = 1, show.sig = TRUE)
```

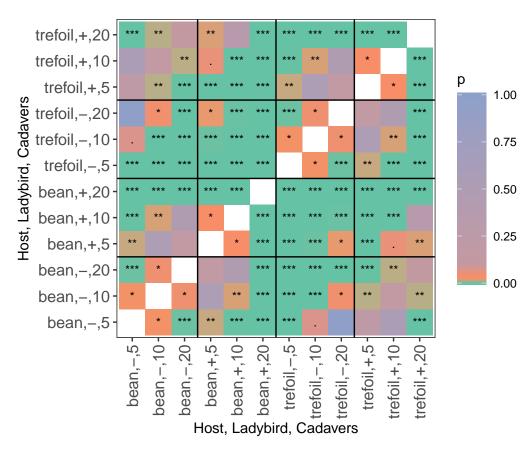


Figure 2. The p-values for each of the pairwise comparisons of the estimated marginal means for logit(P), where P is the proportion of live aphids that were infected, for two Hosts, two Ladybird levels and three Cadaver levels

# options(width = 90) diffs\$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
                          0.4006211 0.0000000
                                                0.2747378 -0.1464657 -1.2234801
              0.85902408
bean,+,5
              0.58428627
                          0.1258833 - 0.2747378 0.0000000 - 0.4212036 - 1.4982179
              1.00548982 \quad 0.5470869 \quad 0.1464657 \quad 0.4212036 \quad 0.0000000 \quad -1.0770144
bean,+,10
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
trefoil,+,5 -0.28491385 -0.7433168 -1.1439379 -0.8692001 -1.2904037 -2.3674180
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
bean,-,10
               1.3276031
                            0.8692001
                                         0.46857901
                                                      0.7433168
                                                                    0.3221133
                                                                                -0.7549011
bean,-,20
               1.7282242
                            1.2698212
                                        0.86920012
                                                      1.1439379
                                                                    0.7227344
                                                                                -0.3542800
bean,+,5
               1.4534864
                            0.9950834
                                         0.59446231
                                                      0.8692001
                                                                    0.4479966
                                                                                -0.6290178
bean,+,10
               1.8746899
                            1.4162870
                                         1.01566586
                                                      1.2904037
                                                                    0.8692001
                                                                                -0.2078143
               2.9517043
                            2.4933014
                                         2.09268024
                                                      2.3674180
                                                                   1.9462145
                                                                                 0.8692001
bean, +, 20
               0.000000
trefoil,-,5
                           -0.4584030
                                        -0.85902408
                                                     -0.5842863
                                                                   -1.0054898
                                                                                -2.0825042
trefoil,-,10
               0.4584030
                            0.0000000
                                        -0.40062111
                                                     -0.1258833
                                                                   -0.5470869
                                                                                -1.6241012
trefoil,-,20
               0.8590241
                            0.4006211
                                         0.0000000
                                                      0.2747378
                                                                   -0.1464657
                                                                                -1.2234801
trefoil,+,5
               0.5842863
                            0.1258833
                                        -0.27473781
                                                      0.0000000
                                                                   -0.4212036
                                                                                -1.4982179
               1.0054898
                            0.5470869
                                                                   0.0000000
                                                                                -1.0770144
trefoil,+,10
                                         0.14646574
                                                      0.4212036
trefoil,+,20
               2.0825042
                            1.6241012
                                         1.22348012
                                                      1.4982179
                                                                    1.0770144
                                                                                 0.0000000
```

# options(width = 90) print(diffs\$sed)

```
bean,-,5 bean,-,10 bean,-,20 bean,+,5 bean,+,10 bean,+,20 trefoil,-,5
bean, -, 5
                    NA 0.1945600 0.1945600 0.1945600 0.1945600 0.1945600
                                                                            0.1123293
             0.1945600
                              NA 0.1945600 0.1945600 0.1945600 0.1945600
bean, -, 10
                                                                            0.2246586
             0.1945600 0.1945600
                                         NA 0.1945600 0.1945600 0.1945600
bean,-,20
                                                                            0.2246586
bean,+,5
             0.1945600 0.1945600 0.1945600
                                                   NA 0.1945600 0.1945600
                                                                            0.2246586
             0.1945600 0.1945600 0.1945600 0.1945600
                                                             NA 0.1945600
                                                                            0.2246586
bean,+,10
             0.1945600 0.1945600 0.1945600 0.1945600 0.1945600
bean,+,20
                                                                       NA
                                                                            0.2246586
trefoil,-,5 0.1123293 0.2246586 0.2246586 0.2246586 0.2246586 0.2246586
                                                                                   NA
trefoil,-,10 0.2246586 0.1123293 0.2246586 0.2246586 0.2246586 0.2246586
                                                                            0.1945600
trefoil,-,20 0.2246586 0.2246586 0.1123293 0.2246586 0.2246586 0.2246586
                                                                            0.1945600
trefoil,+,5 0.2246586 0.2246586 0.2246586 0.1123293 0.2246586 0.2246586
                                                                            0.1945600
trefoil,+,10 0.2246586 0.2246586 0.2246586 0.1123293 0.2246586
                                                                            0.1945600
trefoil,+,20 0.2246586 0.2246586 0.2246586 0.2246586 0.2246586 0.1123293
                                                                            0.1945600
             trefoil,-,10 trefoil,-,20 trefoil,+,5 trefoil,+,10 trefoil,+,20
bean, -,5
                0.2246586
                             0.2246586
                                         0.2246586
                                                       0.2246586
                                                                    0.2246586
bean,-,10
                0.1123293
                             0.2246586
                                         0.2246586
                                                       0.2246586
                                                                    0.2246586
bean, -, 20
                0.2246586
                             0.1123293
                                          0.2246586
                                                       0.2246586
                                                                    0.2246586
bean,+,5
                0.2246586
                             0.2246586
                                          0.1123293
                                                       0.2246586
                                                                    0.2246586
                0.2246586
                             0.2246586
                                          0.2246586
                                                       0.1123293
                                                                    0.2246586
bean,+,10
bean,+,20
                0.2246586
                             0.2246586
                                          0.2246586
                                                       0.2246586
                                                                    0.1123293
trefoil,-,5
                0.1945600
                             0.1945600
                                          0.1945600
                                                       0.1945600
                                                                    0.1945600
trefoil,-,10
                             0.1945600
                                          0.1945600
                                                       0.1945600
                                                                    0.1945600
                       NA
trefoil,-,20
                0.1945600
                                          0.1945600
                                                       0.1945600
                                                                    0.1945600
                                    NA
                                                       0.1945600
                                                                    0.1945600
trefoil,+,5
                0.1945600
                             0.1945600
                                                 NA
trefoil,+,10
                             0.1945600
                                                                    0.1945600
                0.1945600
                                          0.1945600
                                                              NA
trefoil,+,20
                0.1945600
                             0.1945600
                                          0.1945600
                                                       0.1945600
                                                                           NA
```

# Perform the analysis with just the 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 estimated marginal means for the chosen model are obtained.

```
m.sig.lm <- lm(logitP ~ Run + Cadavers*Ladybird + Host,
                         data=Ladybird.dat)
(aov.tab <- anova(m.sig.lm))</pre>
Analysis of Variance Table
Response: logitP
                  Df Sum Sq Mean Sq F value
                                                 Pr(>F)
Run
                   1 0.0677 0.0677 0.3029
                                                0.58398
Cadavers
                   2 17.0274 8.5137 38.1160 1.255e-11
Ladybird
                   1 11.0907 11.0907 49.6531 1.542e-09
Host
                   1 13.5992 13.5992 60.8836 7.179e-11
Cadavers:Ladybird 2 1.7349 0.8675 3.8836
Residuals
                  64 14.2952 0.2234
HCL.emm <- emmeans::emmeans(m.sig.lm, specs = ~ Host:Cadavers:Ladybird)</pre>
HCL.preds <- summary(HCL.emm)</pre>
den.df <- min(HCL.preds$df)</pre>
HCL.vcov <- vcov(HCL.emm)</pre>
HCL.preds <- as.predictions.frame(HCL.preds, predictions = "emmean",</pre>
                                  se = "SE", interval.type = "CI",
                                  interval.names = c("lower.CL", "upper.CL"))
diffs.red <- allDifferences(predictions = HCL.preds, classify = "Host:Ladybird:Cadavers",</pre>
                            vcov = HCL.vcov, tdf = den.df)
diffs.red <- redoErrorIntervals(diffs, error.intervals = "halfLeast",</pre>
                                LSDtype = "factor.combination", LSDby = "Host")
Joining with 'by = join by(fac.comb)'
Joining with 'by = join_by(Host)'
options(width = 90)
print(diffs.red$sed)
              bean, -, 5 bean, -, 10 bean, -, 20 bean, +, 5 bean, +, 10 bean, +, 20 trefoil, -, 5
bean, -, 5
                    NA 0.1945600 0.1945600 0.1945600 0.1945600 0.1945600
                                                                             0.1123293
                              NA 0.1945600 0.1945600 0.1945600 0.1945600
bean,-,10
             0.1945600
                                                                             0.2246586
bean,-,20
             0.1945600 0.1945600
                                         NA 0.1945600 0.1945600 0.1945600
                                                                             0.2246586
bean,+,5
             0.1945600 0.1945600 0.1945600
                                                   NA 0.1945600 0.1945600
                                                                             0.2246586
             0.1945600 0.1945600 0.1945600 0.1945600
bean,+,10
                                                             NA 0.1945600
                                                                             0.2246586
bean,+,20
             0.1945600 0.1945600 0.1945600 0.1945600 0.1945600
                                                                             0.2246586
trefoil,-,5 0.1123293 0.2246586 0.2246586 0.2246586 0.2246586 0.2246586
                                                                                    NA
trefoil,-,10 0.2246586 0.1123293 0.2246586 0.2246586 0.2246586 0.2246586
                                                                             0.1945600
trefoil,-,20 0.2246586 0.2246586 0.1123293 0.2246586 0.2246586 0.2246586
                                                                             0.1945600
trefoil,+,5 0.2246586 0.2246586 0.1123293 0.2246586 0.2246586
                                                                             0.1945600
trefoil,+,10 0.2246586 0.2246586 0.2246586 0.1123293 0.2246586
                                                                             0.1945600
trefoil,+,20 0.2246586 0.2246586 0.2246586 0.2246586 0.2246586 0.1123293
                                                                             0.1945600
```

trefoil,-,10 trefoil,-,20 trefoil,+,5 trefoil,+,10 trefoil,+,20

bean,-,5	0.2246586	0.2246586	0.2246586	0.2246586	0.2246586
bean,-,10	0.1123293	0.2246586	0.2246586	0.2246586	0.2246586
bean,-,20	0.2246586	0.1123293	0.2246586	0.2246586	0.2246586
bean,+,5	0.2246586	0.2246586	0.1123293	0.2246586	0.2246586
bean,+,10	0.2246586	0.2246586	0.2246586	0.1123293	0.2246586
bean,+,20	0.2246586	0.2246586	0.2246586	0.2246586	0.1123293
trefoil,-,5	0.1945600	0.1945600	0.1945600	0.1945600	0.1945600
trefoil,-,10	NA	0.1945600	0.1945600	0.1945600	0.1945600
trefoil,-,20	0.1945600	NA	0.1945600	0.1945600	0.1945600
trefoil,+,5	0.1945600	0.1945600	NA	0.1945600	0.1945600
trefoil,+,10	0.1945600	0.1945600	0.1945600	NA	0.1945600
trefoil,+,20	0.1945600	0.1945600	0.1945600	0.1945600	NA

# References

Brien, C. J. (2025a) asremlPlus: Augments ASReml-R in fitting mixed models and packages generally in exploring prediction differences. Version 4.4.50. https://cran.r-project.org/package=asremlPlus/ or http://chris.brien.name/rpackages/.

Brien, C. J. (2024b) dae: Functions useful in the design and ANOVA of experiments. Version 3.2.30. https://cran.r-project.org/package=dae/ or http://chris.brien.name/rpackages/.

Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. B. (2017) lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, 82, 1–26.

Lenth, R. V., Banfa, B., Bolker, B., Buerkner, P., Giné-Vázquez, I., Herve, M. J., Jung, M., Love, J., Miguez, F., Piaskowski, J., Riebl, H., & Singmann, H. (2025) emmeans: *Estimated Marginal Means, aka Least-Squares Means*. Version 1.11.0. https://cran.r-project.org/package=emmeans/.

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 (2025) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://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.