

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] '4.1.0.130'

suppressMessages(library(asremlPlus))
packageVersion("asremlPlus")

## [1] '4.2.25'

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

## [1] '3.1.32'

options(width = 95, show.signif.stars = FALSE)
```

Get data available in `asremlPlus`

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

Do an ANOVA of logits

```
Ladybird.aov <- aov(logitP ~ Host*Cadavers*Ladybird + Error(Run/Plant),
                  data=Ladybird.dat)
summary(Ladybird.aov)
```

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
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

```
m <- asreml(logitP ~ Host*Cadavers*Ladybird,
            random = ~ Run,
            residual = ~ Run:Plant,
            data = Ladybird.dat)
```

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Model fitted using the gamma parameterization.

ASReml 4.1.0 Wed Nov 11 10:56:46 2020

	LogLik	Sigma2	DF	wall	cpu
1	3.07130	0.226239	60	10:56:46	0.0 (1 restrained)
2	3.64260	0.226912	60	10:56:46	0.0 (1 restrained)
3	3.71250	0.227106	60	10:56:46	0.0 (1 restrained)
4	3.71721	0.227121	60	10:56:46	0.0 (1 restrained)
5	3.71751	0.227122	60	10:56:46	0.0 (1 restrained)

Warning in asreml(logitP ~ Host * Cadavers * Ladybird, random = ~Run, residual = ~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 Wed Nov 11 10:56:46 2020

	LogLik	Sigma2	DF	wall	cpu
1	3.07130	0.226239	60	10:56:46	0.0 (1 restrained)
2	3.64260	0.226912	60	10:56:46	0.0 (1 restrained)
3	3.80283	0.227454	60	10:56:46	0.0 (1 restrained)
4	3.83949	0.233418	60	10:56:46	0.0
5	3.95568	0.230971	60	10:56:46	0.0
6	3.97333	0.230035	60	10:56:46	0.0
7	3.97405	0.229835	60	10:56:46	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")
```

```
#### 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	1	59	59.17	0.0000
Cadavers	2	59	37.04	0.0000
Ladybird	1	59	48.26	0.0000
Host:Cadavers	2	59	0.67	0.5158
Host:Ladybird	1	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.

```
Ladybird.pstr <- pstructure(formula = ~ Host*Cadavers*Ladybird,
                           data = Ladybird.dat)
HCL.marg <- marginality(Ladybird.pstr)
print(HCL.marg)
```

	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
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

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

```
Calculating denominator DF
Calculating denominator DF
Calculating denominator DF
Calculating denominator DF
Calculating denominator DF
```

```
current.asrt <- chosen$asrtests.obj
print(current.asrt, which = "test", omit.columns = c("AIC", "BIC"))
```

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

	terms	DF	denDF	p	action
1	Host:Cadavers:Ladybird	2	59	0.6493	Nonsignificant
2	Cadavers:Ladybird	2	59	0.0287	Significant
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 `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)))
```

```
~Cadavers:Ladybird + Host
```

Obtain predictions under the chosen model and form an alldiffs object

```
diffs <- predictPlus(current.asrt$asreml.obj,  
                    classify = "Host:Ladybird:Cadavers",  
                    linear.transformation = ~Cadavers:Ladybird + Host,  
                    wald.tab = current.asrt$wald.tab,  
                    error.intervals = "halfLeast",  
                    meanLSD.type = "factor.combination", LSDby = "Host",  
                    tables = "predictions")
```

```
#### 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
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.halfLeastSignificant.limit	est.status
1	-1.7996454	Estimable
2	-1.3412425	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
9	-1.8098215	Estimable
10	-2.0845593	Estimable
11	-1.6633557	Estimable
12	-0.5863414	Estimable

LSD values

minimum LSD = 0.3916233 0.3916233

mean LSD = 0.3916233 0.3916233

maximum LSD = 0.3916233 0.3916233

(sed range / mean sed = 5.67e-16 7.09e-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 of 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, calculate predictions to check first and then transform to conform to chosen model

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

diffs <- linTransform(diffs.full, linear.transformation = ~Cadavers:Ladybird + Host,
  wald.tab = current.asrt$wald.tab,
  error.intervals = "halfLeast",
  meanLSD.type = "factor.combination", LSDby = "Host",
  tables = "predictions")
```

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 hypertextable 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
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.halfLeastSignificant.limit			est.status		
1	-1.7996454			Estimable		
2	-1.3412425			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		
9	-1.8098215			Estimable		
10	-2.0845593			Estimable		
11	-1.6633557			Estimable		
12	-0.5863414			Estimable		

LSD values

minimum LSD = 0.3916233 0.3916233

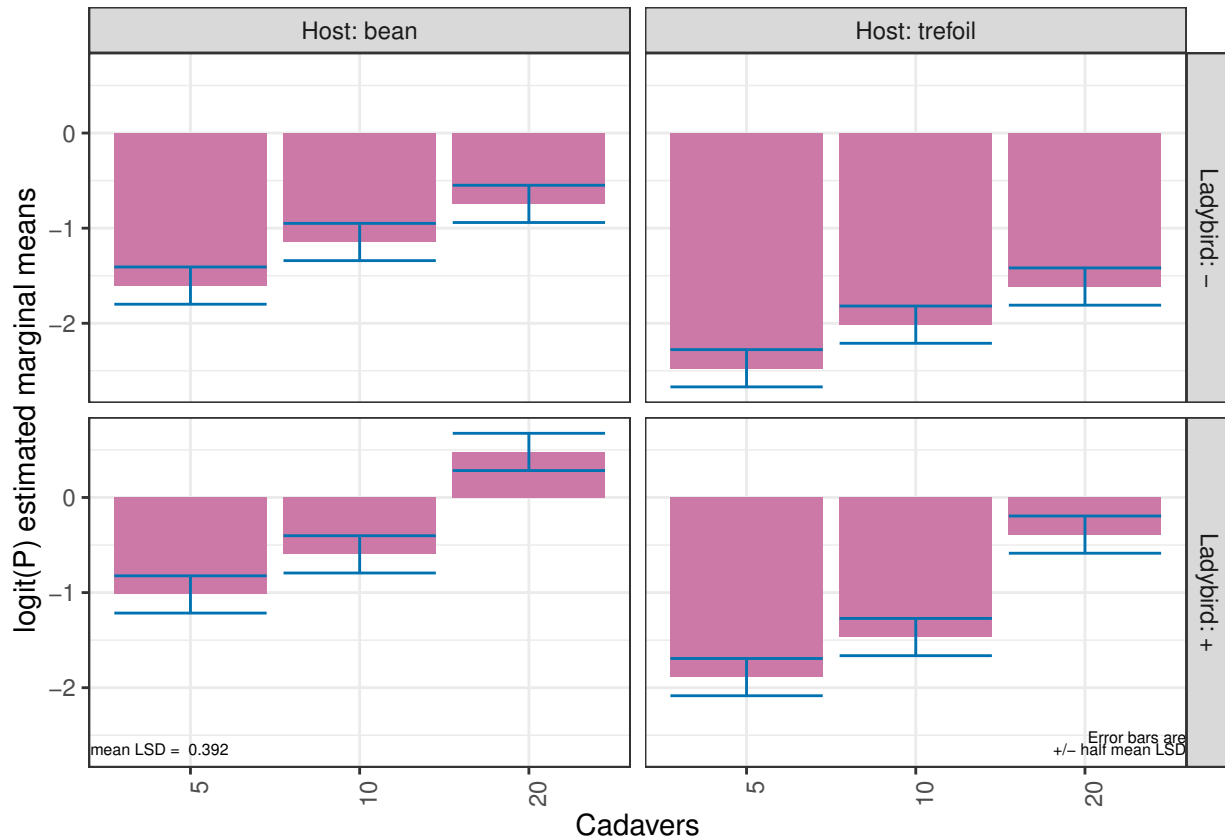
mean LSD = 0.3916233 0.3916233

maximum LSD = 0.3916233 0.3916233

(sed range / mean sed = 5.67e-16 7.09e-16)

Plot the predictions

```
plotPredictions(diffs$predictions, y = "predicted.value",
  y.title = "logit(P) estimated marginal means",
  classify = "Host:Ladybird:Cadavers",
  error.intervals = "halfLeast",
  ggplotFuncs = list(facet_grid(Ladybird ~ Host,
    labeller = label_both)))
```



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 $\pm 0.5LSD$ 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`.

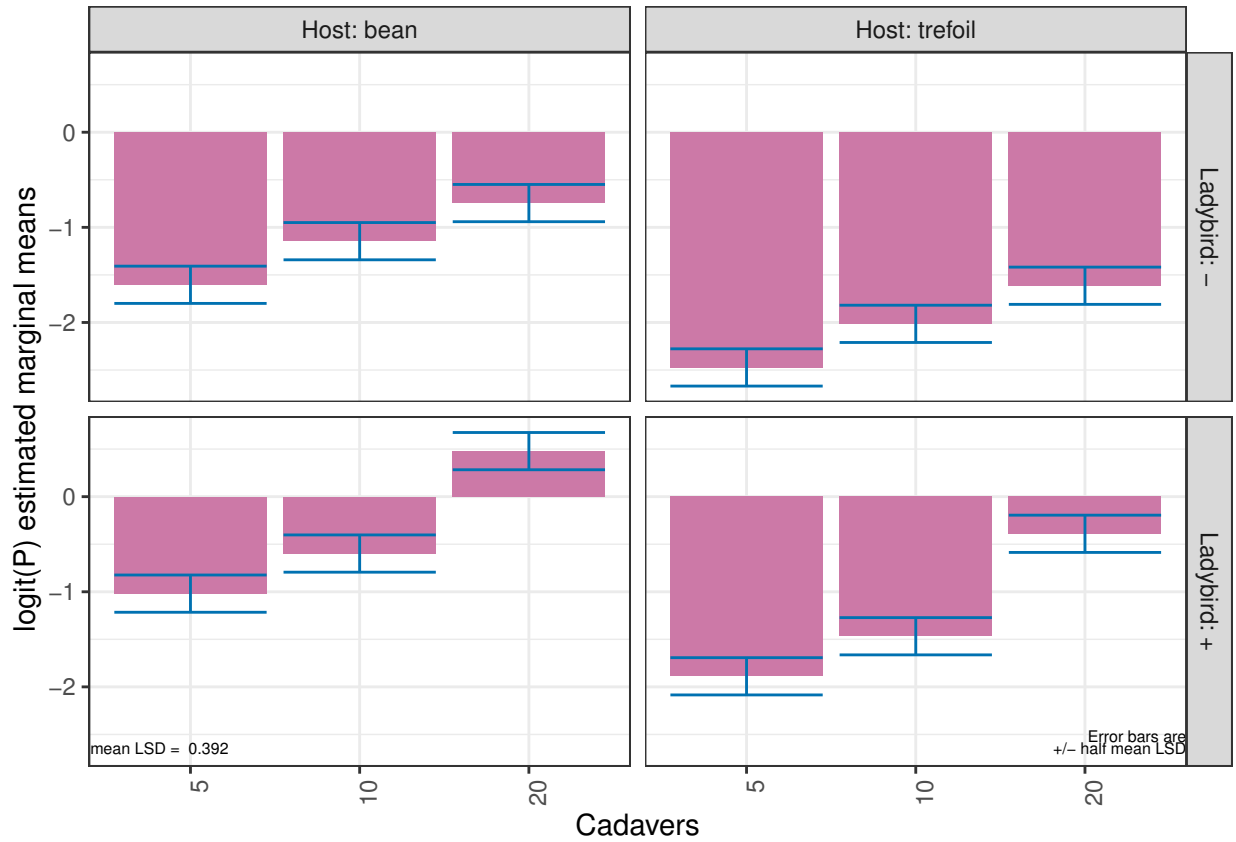
```
titl <- "logit(P) estimated marginal means"
names(titl) <- "logitP"
diffs <- predictPresent(current.asrt$asreml.obj,
  terms = "Host:Ladybird:Cadavers",
```



```

linear.transformation = ~Cadavers:Ladybird + Host,
titles = titl,
wald.tab = current.asrt$wald.tab,
error.intervals = "halfLeast",
meanLSD.type = "factor.combination", LSDby = "Host",
tables = "none",
ggplotFuncs = list(facet_grid(Ladybird ~ Host,
                             labeller = label_both)))

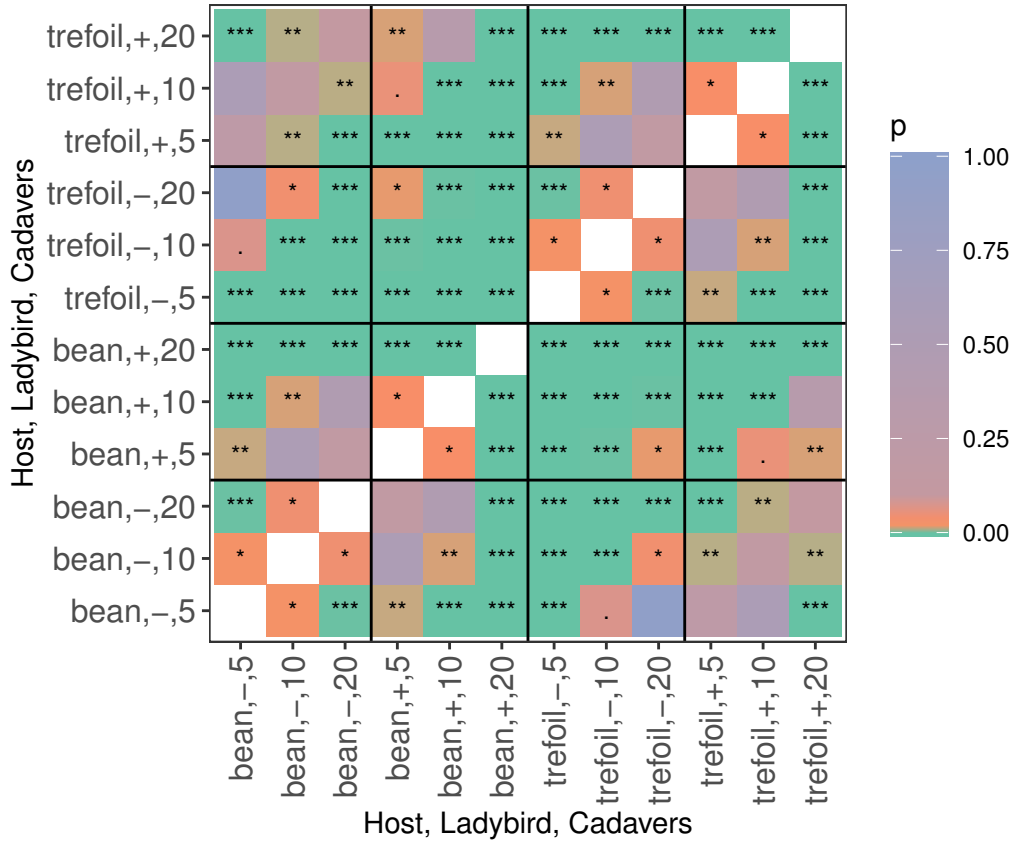
```



```

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	0.0000000	-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	0.85902408	0.4006211	0.0000000	0.2747378	-0.1464657	-1.2234801
bean+,5	0.58428627	0.1258833	-0.2747378	0.0000000	-0.4212036	-1.4982179
bean+,10	1.00548982	0.5470869	0.1464657	0.4212036	0.0000000	-1.0770144
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
bean+,20	2.9517043	2.4933014	2.09268024	2.3674180	1.9462145	0.8692001
trefoil,-,5	0.0000000	-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
trefoil,+,10   1.0054898    0.5470869    0.14646574    0.4212036    0.0000000    -1.0770144
trefoil,+,20   2.0825042    1.6241012    1.22348012    1.4982179    1.0770144    0.0000000

```

```

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 0.1957142          NA 0.1957142 0.1957142 0.1957142 0.1957142 0.2259913
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          NA 0.1957142 0.2259913
bean,+,20 0.1957142 0.1957142 0.1957142 0.1957142 0.1957142          NA 0.2259913
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 0.2259913 0.1129957 0.2259913 0.2259913 0.1957142
trefoil,+,10 0.2259913 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 0.2259913 0.1129957 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
bean,+,20 0.2259913 0.2259913 0.2259913 0.2259913 0.1129957
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          NA 0.1957142 0.1957142 0.1957142
trefoil,+,5 0.1957142 0.1957142          NA 0.1957142 0.1957142
trefoil,+,10 0.1957142 0.1957142 0.1957142          NA 0.1957142
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 estimated marginal means for the chosen model are obtained.

```

ns.terms <- current.asrt$test.summary$terms[current.asrt$test.summary$action == "Nonsignificant"]
red.asrt <- changeTerms(current.asrt, dropFixed = paste(ns.terms, collapse = "+"))

```

Calculating denominator DF

Calculating denominator DF

```
summary(red.asrt$asreml.obj)$varcomp
```

```

          component  std.error  z.ratio bound %ch
Run          -0.004327123 0.002802858 -1.543825    U 0.8
Run:Plant!R  0.223431515 0.039503521  5.655990    P 0.0

```

```
print(red.asrt, which = "pseudoanova")
```

```
#### 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	1	64	60.88	0.0000
Cadavers	2	64	38.12	0.0000
Ladybird	1	64	49.65	0.0000
Cadavers:Ladybird	2	64	3.88	0.0256

```
diffs.red <- predictPlus(red.asrt$asreml.obj,
  classify = "Host:Ladybird:Cadavers",
  wald.tab = current.asrt$wald.tab,
  error.intervals = "halfLeast",
  meanLSD.type = "factor.combination", LSDby = "Host",
  tables = "predictions")
```

Predictions for logitP from Host:Ladybird:Cadavers

Notes:

- The 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
1	bean	-	5	-1.6038338	0.1398332
2	bean	-	10	-1.1454308	0.1398332
3	bean	-	20	-0.7448097	0.1398332
4	bean	+	5	-1.0195475	0.1398332
5	bean	+	10	-0.5983440	0.1398332
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	-1.4107942	-1.7968734	Estimable
2	-0.9523913	-1.3384704	Estimable
3	-0.5517702	-0.9378493	Estimable
4	-0.8265080	-1.2125871	Estimable
5	-0.4053044	-0.7913835	Estimable
6	0.6717100	0.2856308	Estimable
7	-2.2799944	-2.6660735	Estimable
8	-1.8215914	-2.2076705	Estimable
9	-1.4209703	-1.8070494	Estimable
10	-1.6957081	-2.0817872	Estimable
11	-1.2745045	-1.6605837	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	0.1929435	NA	0.1929435	0.1929435	0.1929435	0.1929435	0.2227920
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
bean,+,20	0.1929435	0.1929435	0.1929435	0.1929435	0.1929435	NA	0.2227920
trefoil,-,5	0.1113960	0.2227920	0.2227920	0.2227920	0.2227920	0.2227920	NA
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	0.2227920	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		
bean,-,10	0.1113960	0.2227920	0.2227920	0.2227920	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		
bean,+,10	0.2227920	0.2227920	0.2227920	0.1113960	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	NA	0.1929435	0.1929435	0.1929435	0.1929435		
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		
trefoil,+,20	0.1929435	0.1929435	0.1929435	0.1929435	NA		

References

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/>.

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.