Using asremlPlus, in conjunction with asreml, to do a linear mixed model analysis of a wheat experiment using hypothesis tests

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This vignette shows how to use asremlPlus (Brien, 2024), in conjunction with asreml (Butler et al., 2020), to employ hypothesis tests to select the terms to be included in a mixed model for an experiment that involves spatial variation. It also illustrates diagnostic checking and prediction production and presentation for this experiment. Here, asremlPlus and asreml are packages for the R Statistical Computing environment (R Core Team, 2024).

It is divided into the following main sections:

- 1. Set up the maximal model for this experiment
- 2. Perform a series of hypothesis tests to select a linear mixed model for the data
- 3. Diagnostic checking using residual plots and variofaces
- 4. Prediction production and presentation

## 1. Set up the maximal model for this experiment

```
library(knitr)
opts_chunk$set("tidy" = FALSE, comment = NA)
suppressMessages(library(asrem1, quietly=TRUE))

## Offline License checked out Mon Oct 28 10:52:25 2024

packageVersion("asrem1")

## [1] '4.2.0.332'
suppressMessages(library(asrem1Plus))
packageVersion("asrem1Plus")

## [1] '4.4.40'
suppressMessages(library(qqplotr, quietly=TRUE))
options(width = 100)
```

#### Get data available in asremlPlus

The data are from a 1976 spring wheat experiment and are taken from Gilmour et al. (1995). An analysis is presented in the asrem1 manual by Butler et al. (2020, Section 7.6), although they suggest that it is a barley experiment.

```
data(Wheat.dat)
```

#### Fit the maximal model

In the following a model is fitted that has the terms that would be included for a balanced lattice. In addition, a term WithinColPairs has been included to allow for extraneous variation arising between pairs of adjacent lanes. Also, separable ar1 residual autocorrelation has been included. This model represents the maximal anticipated model,

```
ASReml Version 4.2 28/10/2024 10:52:25
         LogLik
                        Sigma2
                                   DF
                                          wall
       -724.1213
                      23034.14
                                  124
                                        10:52:25
1
2
      -717.4149
                      9206.931
                                  124
                                        10:52:25
                                                  ( 2 restrained)
 3
      -694.8752
                      26492.99
                                  124
                                        10:52:25
                                                  ( 2 restrained)
      -694.1600
 4
                      33101.80
                                  124
                                        10:52:25
                                                  ( 1 restrained)
 5
      -692.0020
                      36912.26
                                  124
                                        10:52:25
                                                  ( 1 restrained)
 6
      -691.7892
                                  124
                                        10:52:25
                                                  ( 2 restrained)
                      46701.51
 7
      -691.8336
                      46208.51
                                  124
                                        10:52:25
                                                  ( 1 restrained)
8
       -691.7749
                                        10:52:25
                      47698.26
                                  124
       -691.7711
                      47041.85
                                  124
                                        10:52:25
```

Warning in asreml(yield ~ WithinColPairs + Variety, random = ~Rep/(Row + : Some components changed by more than 1% on the last iteration

The warning from asreml is probably due to a bound term.

#### Initialize a testing sequence by loading the current fit into an asrtests object

A label and the information criteria based on the full likelihood (Verbyla, 2019) are included in the test.summary stored in the asrtests object.

Warning in infoCriteria.asreml(asreml.obj, IClikelihood = ic.lik, bound.exclusions = bound.exclusions):
Rep

Warning in asreml(fixed = yield ~ WithinColPairs + Variety, random = ~Rep/(Row + : Log-likelihood not converged

#### Check for and remove any boundary terms

```
current.asrt <- rmboundary(current.asrt, IClikelihood = "full")</pre>
Warning in infoCriteria.asreml(asreml.obj, IClikelihood = ic.lik): The following bound terms were disco
ASReml Version 4.2 28/10/2024 10:52:26
          LogLik
                        Sigma2
                                  DF
                                          wall
                      47071.42
       -691.7710
                                  124
                                       10:52:26
Warning in asreml(fixed = yield ~ WithinColPairs + Variety, random = ~Rep/(Row + : Log-likelihood
not converged
summary(current.asrt$asreml.obj)$varcomp
                                                   z.ratio bound %ch
                         component
                                      std.error
Rep:Row
                      4.293282e+03 3.199458e+03 1.3418779
                                                               P 0.0
Rep:Column
                      1.575689e+02 1.480357e+03 0.1064398
                                                               P 0.7
units
                      5.742689e+03 1.652457e+03 3.4752438
                                                               P 0.0
                      4.706787e+04 2.515832e+04 1.8708669
Row:Column!R
                                                               P 0.0
Row:Column!Row!cor
                      7.920301e-01 1.014691e-01 7.8056280
                                                               U 0.0
Row:Column!Column!cor 8.799559e-01 7.370402e-02 11.9390486
                                                               U 0.0
print(current.asrt, which = "testsummary")
```

#### Sequence of model investigations

(If a row has NA for p but not denDF, DF and denDF relate to fixed and variance parameter numbers)

```
terms DF denDF p AIC BIC action 1 Maximal model 26 6 NA 1646.129 1742.47 Starting model 2 Rep 1 NA NA 1646.129 1742.47 Boundary
```

Rep has been removed because it has been constrained to zero. Following the recommendation of Littel et al. (2006, p. 150), the bound on all variance components is set to unconstrained (U) using setvariances.asreml so as to avoid bias in the estimate of the residual variance. Alternatively, one could move Rep to the fixed model.

#### Unbind Rep, Row and Column components and reload into an asrtests object

```
ASReml Version 4.2 28/10/2024 10:52:27
                                        wall
         LogLik
                      Sigma2
                                DF
                                124
                                     10:52:27
1
      -724.1213
                     23034.14
2
      -717.4149
                     9206.931 124 10:52:27 ( 2 restrained)
                              124 10:52:27 ( 2 restrained)
 3
      -694.8752
                     26492.99
 4
      -693.9744
                    33129.65 124 10:52:27 ( 1 restrained)
5
      -692.8856
                   39662.12 124 10:52:27
                  53103.83 124 10:52:27
6
      -691.4276
                  48092.17 124
7
      -691.2387
                                     10:52:27
8
                  47278.94 124
                                     10:52:27
      -691.1808
9
      -691.1710
                    46850.98 124 10:52:27
      -691.1700
                    46690.46 124 10:52:27
10
Warning in asreml(fixed = yield ~ WithinColPairs + Variety, random = ~Rep/(Row + : Some components
changed by more than 1\% on the last iteration
current.asrt <- as.asrtests(current.asr, wald.tab = NULL, test.summary = current.asrt$test.summary,</pre>
                          IClikelihood = "full", label = "Max model & Unbound components")
current.asrt <- rmboundary(current.asrt)</pre>
summary(current.asrt$asreml.obj)$varcomp
                         component
                                                  z.ratio bound %ch
                                     std.error
                    -2458.3485841 1.197491e+03 -2.0529167
                                                             U 0.0
Rep
                     5008.7151486 3.401335e+03 1.4725732
                                                             U 0.0
Rep:Row
Rep:Column
                      916.4641198 1.699576e+03 0.5392309
                                                             U 0.2
units
                     5959.0220817 1.609649e+03 3.7020634
                                                             P 0.0
Row: Column! R.
                     46637.6303429 2.724392e+04 1.7118545
                                                           P 0.0
Row:Column!Row!cor
                         0.8150590 1.000281e-01 8.1483012
                                                             U 0.0
Row:Column!Column!cor
                        0.8856824 7.492514e-02 11.8208968
                                                             U 0.0
print(current.asrt, which = "testsummary")
#### Sequence of model investigations
(If a row has NA for p but not denDF, DF and denDF relate to fixed and variance parameter numbers)
                          terms DF denDF p
                                                AIC
                                                        BIC
                                                                    action
                  Maximal model 26 6 NA 1646.129 1742.470 Starting model
1
                                     NA NA 1646.129 1742.470
                           Rep 1
3 Max model & Unbound components 26
                                     7 NA 1647.193 1746.544 Starting model
print(current.asrt, which = "pseudoanova")
```

#### Pseudo-anova table for fixed terms

Wald tests for fixed effects.

#### Response: yield

```
Df denDF F.inc Pr
(Intercept) 1 1.7 153.500 0.0115
WithinColPairs 1 15.6 2.545 0.1307
Variety 24 76.1 10.110 0.0000
```

Now the Rep component estimate is negative.

The test.summary output has been extended, by supplying the previous test.summary to as.asrtests, to show that there is a new starting model. The pseudo-anova table shows that Varieties are highly significant (p < 0.001)

# 2. Perform a series of hypothesis tests to select a linear mixed model for the data

The hypothesis tests in this section are Wald tests for fixed terms, with denominator degrees of freedom calculated using the Kenward-Rogers adjustment (Kenward and Rogers (1997), and Restricted Maximum Likelihood Ratio Tests (REMLRT) for random terms.

#### Check the term for within Column pairs (a post hoc factor)

The information criteria based on the full likelihood (Verbyla, 2019) is also included in the test.summary stored in the asrtests object.

WARN [2024-10-28 10:52:29] Some components changed by more than 1% on the last iteration

Warning in asreml(fixed = yield  $\sim$  Variety, random =  $\sim$ Rep/(Row + Column) + : Some components changed by more than 1% on the last iteration

WARN [2024-10-28 10:52:29] Some components changed by more than 1% on the last iteration

Warning in asreml(fixed = yield ~ Variety, random = ~Rep/(Row + Column) + : Some components changed by more than 1% on the last iteration

```
print(current.asrt)
```

#### Summary of the fitted variance parameters

```
componentstd.errorz.ratiobound%chRep-2391.94899391.194581e+03-2.0023338U 0.4Rep:Row5035.53110543.406006e+031.4784269U 0.3Rep:Column761.95356221.612103e+030.4726458U 1.2units5933.21337941.610805e+033.6833848P 0.1
```

```
Row:Column!R 45970.8383027 2.635124e+04 1.7445415 P 0.0 Row:Column!Row!cor 0.8101615 9.995498e-02 8.1052641 U 0.1 Row:Column!Cor 0.8846970 7.503039e-02 11.7911827 U 0.0
```

#### Pseudo-anova table for fixed terms

Wald tests for fixed effects. Response: yield

```
Df denDF F.inc Pr (Intercept) 1 1.7 158.90 0.0112 Variety 24 76.8 10.27 0.0000
```

#### Sequence of model investigations

(If a row has NA for p but not denDF, DF and denDF relate to fixed and variance parameter numbers)

```
terms DF denDF
                                                      AIC
                                                                BIC
                                                                            action
                                               р
                   Maximal model 26
                                      6.0
                                              NA 1646.129 1742.470 Starting model
                             Rep 1
                                       NA
                                              NA 1646.129 1742.470
                                                                          Boundary
3 Max model & Unbound components 26
                                      7.0
                                              NA 1647.193 1746.544 Starting model
                  WithinColPairs 1 15.6 0.1307 1645.325 1741.666
                                                                           Dropped
```

It is clear in the call to testranfix that the model is being changed by dropping the withinColPairs term, which could also be achieved using update.asreml. However, an asremlPlus model-changing function operates on an asrtests object, that includes an asreml object, and, except for changeTerms.asrtests, results in an asrtests object that may contain the changed model or the supplied model depending on the results of hypothesis tests or comparisons of information criteria. In addition, the result of the test or comparison will be added to a test.summary data.frame stored in the new asrtests object and, if the model was changed, the wald.tab in the new asrtests object will have been updated for the new model.

In this case, as can be seen from the summary of current.asrt after the call, the *p*-value for the withinColPairs was greater than 0.05 and so now the model stored in current.asrt does not include withinColPairs. The wald.tab has been updated for the new model.

#### Test the nugget term

The nugget term represents non-spatial variance, such as random plot and measurement error. It is fitted using the asreml reserved word units.

```
current.asrt <- testranfix(current.asrt, "units", positive=TRUE, IClikelihood = "full")</pre>
```

WARN [2024-10-28 10:52:31] Some components changed by more than 1% on the last iteration

Warning in asreml(fixed = yield ~ Variety, random = ~Rep + Rep:Row + Rep:Column, : Some components changed by more than 1% on the last iteration

WARN [2024-10-28 10:52:31] Some components changed by more than 1% on the last iteration

Warning in asreml(fixed = yield ~ Variety, random = ~Rep + Rep:Row + Rep:Column, : Some components changed by more than 1% on the last iteration

#### Test Row autocorrelation

We begin testing the autocorrelation by dropping the Row autocorrelation. Because of messages about the instability of the fit, iterate.asrtests is used to execute extra iterations of the fitting process.

WARN [2024-10-28 10:52:33] Some components changed by more than 1% on the last iteration

Warning in asreml(fixed = yield ~ Variety, random = ~Rep/(Row + Column) + : Some components changed by more than 1% on the last iteration

WARN [2024-10-28 10:52:33] Some components changed by more than 1% on the last iteration

Warning in asreml(fixed = yield  $\sim$  Variety, random =  $\sim$ Rep/(Row + Column) + : Some components changed by more than 1% on the last iteration

```
current.asrt <- iterate(current.asrt)</pre>
```

## Test Column autocorrelation (depends on whether Row autocorrelation retained)

The function getTestPvalue is used to get the p-value for the Row autocorrelation test. If it is significant then the Column autocorrelation is tested by by dropping the Column autocorrelation, while retaining the Row autocorrelation. Otherwise the model with just Row autocorrelation, whose fit is returned via current.asrt after the test, is compared to one with no autocorrelation.

```
(p <- getTestPvalue(current.asrt, label = "Row autocorrelation"))</pre>
```

[1] 4.676754e-06

Warning in DFdiff(bound.h1, bound.h0, DF = DF, bound.exclusions = bound.exclusions): There were a total The following bound terms occur in only one of the models compared and so were discounted:

Row:Column!Row!cor

#### Output the results

#### print(current.asrt)

#### #### Summary of the fitted variance parameters

	component	std.error	z.ratio	bound	%ch
Rep	-2385.8697551	1.211207e+03	-1.9698276	U	0.0
Rep:Row	5027.7123253	3.415391e+03	1.4720753	U	0.0
Rep:Column	753.5913536	1.609865e+03	0.4681086	U	0.6
units	5920.3547038	1.611274e+03	3.6743304	P	0.0
Row:Column!R	45870.0971595	2.623601e+04	1.7483638	P	0.0
Row:Column!Row!cor	0.8098786	1.001805e-01	8.0841906	U	0.0
Row:Column!Column!cor	0.8845768	7.510598e-02	11.7777144	U	0.0

#### #### Pseudo-anova table for fixed terms

Wald tests for fixed effects.

Response: yield

Df denDF F.inc Pr (Intercept) 1 1.7 159.20 0.0111 Variety 24 76.8 10.27 0.0000

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

(If a row has NA for p but not denDF, DF and denDF relate to fixed and variance parameter numbers)

	terms	DF	${\tt denDF}$	р	AIC	BIC	action
1	Maximal model	. 26	6.0	NA	1646.129	1742.470	Starting model
2	Rep	1	NA	NA	1646.129	1742.470	Boundary
3 Max	model & Unbound components	26	7.0	NA	1647.193	1746.544	Starting model
4	WithinColPairs	: 1	15.6	0.1307	1645.325	1741.666	Dropped
5	units	: 1	NA	0.0006	1645.325	1741.666	Retained
6	Row autocorrelation	1	NA	0.0000	1645.325	1741.666	Unswapped
7	Col autocorrelation	. 2	NA	0.0000	1645.318	1741.658	Unswapped

printFormulae(current.asrt\$asreml.obj)

#### #### Formulae from asreml object

fixed: yield ~ Variety

random: ~ Rep/(Row + Column) + units
residual: ~ ar1(Row):ar1(Column)

```
print(R2adj(current.asrt$asreml.obj, include.which.random = ~ .))
ASReml Version 4.2 28/10/2024 10:52:37
          LogLik
                        Sigma2
                                           wall
       -694.6149
                      45855.31
                                  125
                                         10:52:37
2
       -694.6149
                      45854.06
                                  125
                                         10:52:37
[1] 44.62413
attr(,"fixed")
<environment: 0x000002365a889708>
attr(,"random")
```

The test.summary shows is that the model with Row and without Column autocorrelation failed to converge. The asreml.obj in current.asrt contains the model selected by the selection process, which has been printed using printFormulae.asrtests. It is clear that no changes were made to the variance terms. The adjusted  $R^2$  value shows that the fixed and random terms in the fitted model account for 45% of the total variation in the yield.

### 3. Diagnosing checking using residual plots and variofaces

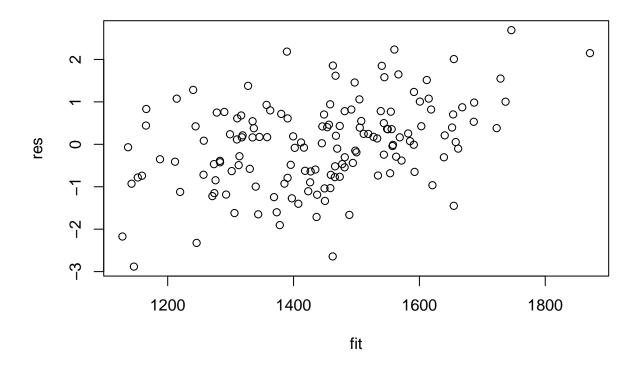
Get current fitted asreml object and update to include standardized residuals

```
current.asr <- current.asrt$asreml.obj</pre>
current.asr <- update(current.asr, aom=TRUE)</pre>
ASReml Version 4.2 28/10/2024 10:52:37
          LogLik
                          Sigma2
                                     DF
                                              wall
1
       -694.6149
                        45855.31
                                     125
                                           10:52:37
 2
       -694.6149
                        45854.06
                                     125
                                           10:52:37
       -694.6149
                        45851.09
                                     125
                                           10:52:37
Wheat.dat$res <- residuals(current.asr, type = "stdCond")</pre>
Wheat.dat$fit <- fitted(current.asr)</pre>
```

#### Do diagnostic checking

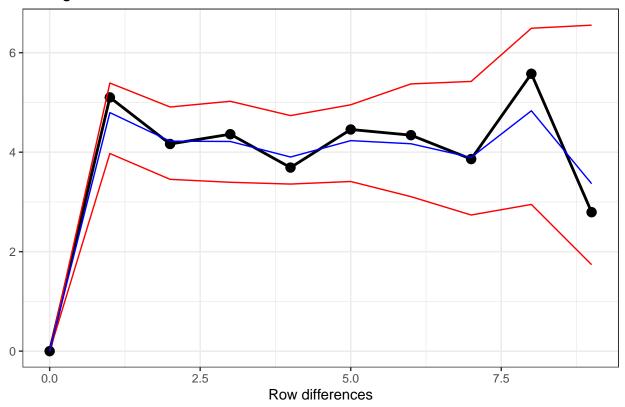
Do residuals-versus-fitted values plot

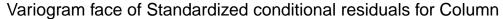
```
with(Wheat.dat, plot(fit, res))
```

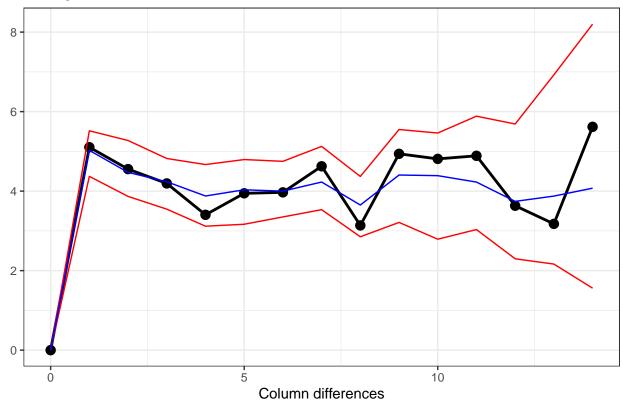


### Plot variofaces

## Variogram face of Standardized conditional residuals for Row





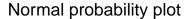


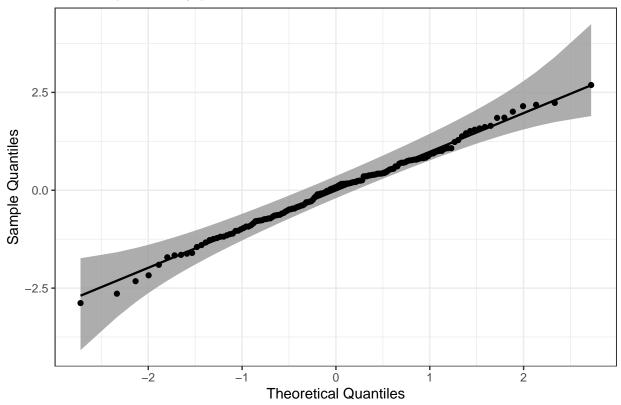
The variofaces are the lag 1 plots of the sample semivariogram with simulated confidence envelopes (Stefanova et al., 2009).

#### Plot normal quantile plot

The plot is obtained using the ggplot function with extensions available from the qqplotr package (Almeida, A., Loy, A. and Hofmann, H., 2023).

```
suppressWarnings(
  ggplot(data = Wheat.dat, mapping = aes(sample = res)) +
    stat_qq_band(bandType = "ts") + stat_qq_line() + stat_qq_point() +
    labs(x = "Theoretical Quantiles", y = "Sample Quantiles",
        title = "Normal probability plot") +
    theme(plot.title = element_text(size = 12, face = "bold")) + theme_bw())
```





## 4. Prediction production and presentation

Get Variety predictions and all pairwise prediction differences and p-values

#### Predictions for yield from Variety

#### 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: Rep,Row,Column,units

- Variety is included in this prediction
- (Intercept) is included in this prediction
- units is ignored in this prediction

	Variatu	prodicted value	atandard .	orror	unnor	halfLeastSignificant.limit
1	variety 10	1168.989		.4768	upper.	1228.315
2	10	1242.750		.8104		1302.076
3	9	1257.137		.9708		1316.463
4	16	1285.718		.9400		1345.045
5	14	1293.716		.9227		1352.853
6	23	1313.653		. 2929		1372.979
7	11	1322.159		. 1964		1381.485
8	7	1374.447		. 2407		1433.773
9	3	1394.070		.4032		1453.775
10	4	1410.980		.1055		1470.306
11	12	1444.557		.6034		1503.883
12	8	1453.396		.5940		1512.723
13	15	1458.383		.4346		1517.709
14	5	1473.782		. 4455		1533.108
15	17	1487.828		. 2896		1547.154
16	6	1498.294		.1189		1557.620
17	21	1517.121		.2262		1576.447
18	2	1520.466		.6322		1579.792
19	24	1533.769		.2995		1593.095
20	18	1541.148		.3664		1600.474
21	25	1575.795		.5142		1635.121
22	22	1610.482		.3281		1669.808
23	13	1610.762	120	.4575		1670.088
24	20	1627.971	120	. 2328		1687.297
25	19	1652.992	120	.3435		1712.318
	lower.ha	alfLeastSignifica	ant.limit	est.st	atus	
1			1109.663	Estim	able	
2			1183.424	Estim	able	
3			1197.811	Estim	able	
4			1226.392	Estim	able	
5			1234.200	Estim	able	
6			1254.327	Estim	able	
7			1262.832	Estim	able	
8			1315.120	Estim	able	
9			1334.743	Estim	able	
10			1351.653	Estim	able	
11			1385.231	Estim	able	
12			1394.070	Estin	able	

1399.057 Estimable

1414.456 Estimable 1428.501 Estimable

1438.968 Estimable

1457.795 Estimable

1461.140 Estimable

1474.443 Estimable

1481.821 Estimable

1516.468 Estimable

1551.156 Estimable

1551.436 Estimable

13 14

15 16

17

18

19

20

21

22

23

14

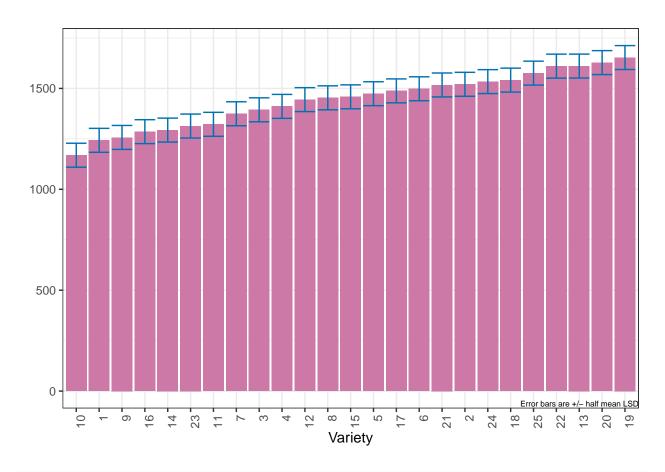
We have set error.intervals to halfLeast so that the limits for so that the limits for each prediction  $\pm$  (0.5 LSD) are calculated. When these are plotted overlapping error bars indicate predictions that are not significant, while those that do not overlap are significantly different (Snee, 1981).

Also set was sortFactor, so that the results would be ordered for the values of the predictions for Variety.

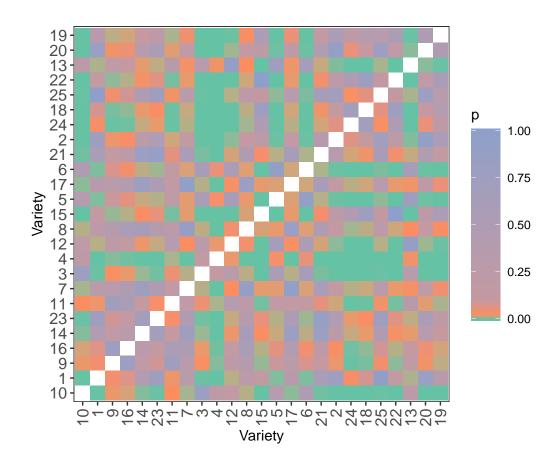
The function predictPlus returns an alldiffs object, a list consisting of the following components:

- predictions: the predictions, their standard errors and error intervals;
- vcov: the variance matrix of the predictions;
- differences: all pairwise differences between the predictions,
- p.differences: p-values for all pairwise differences between the predictions;
- sed: the standard errors of all pairwise differences between the predictions;
- LSD: the mean, minimum and maximum LSDs.

#### Plot the Variety predictions, with halfLSD intervals, and the p-values



plotPvalues(Var.diffs)



#### References

Almeida, A., Loy, A. and Hofmann, H. (2023) qqplotr: Quantile-Quantile plot extensions for 'ggplot2', Version 0.0.6. https://cran.r-project.org/package=qqplotr/ or https://github.com/aloy/qqplotr/.

Brien, C. J. (2024) asremlPlus: Augments ASReml-R in fitting mixed models and packages generally in exploring prediction differences. Version 4.4.40. https://cran.r-project.org/package=asremlPlus/ or http://chris.brien.name/rpackages/.

Butler, D. G., Cullis, B. R., Gilmour, A. R., Gogel, B. J. and Thompson, R. (2023). ASReml-R Reference Manual Version 4.2. VSN International Ltd, https://asreml.kb.vsni.co.uk/.

Gilmour, A. R., Thompson, R., & Cullis, B. R. (1995). Average Information REML: An Efficient Algorithm for Variance Parameter Estimation in Linear Mixed Models. *Biometrics*, **51**, 1440–1450.

Kenward, M. G., & Roger, J. H. (1997). Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics*, **53**, 983-997.

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

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