

# 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(asreml, quietly=TRUE))
```

```
## Offline License checked out Mon Feb 26 11:11:21 2024
```

```
packageVersion("asreml")
```

```
## [1] '4.2.0.276'
```

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

```
## [1] '4.4.29'
```

```
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 `asreml` 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,

```
current.asr <- asreml(yield ~ WithinColPairs + Variety,
  random = ~ Rep/(Row + Column) + units,
  residual = ~ ar1(Row):ar1(Column),
  maxit = 30, data=Wheat.dat)
```

```
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.

```
current.asrt <- as.asrtests(current.asr, NULL, NULL,
  label = "Maximal model", IClkelihood = "full")
```

```
Warning in infoCriteria.asreml(asreml.obj, IClkelihood = 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, IClkelihood = "full")
```

```
Warning in infoCriteria.asreml(asreml.obj, IClkelihood = ic.lik): The following bound terms were discor
Rep
```

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

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

	component	std.error	z.ratio	bound	%ch
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
Row:Column!R	4.706787e+04	2.515832e+04	1.8708669	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	1742.469	Starting model
2	Rep	1	NA	NA	1646.129	1742.469	Boundary

Rep has been removed because it has been constrained to zero. Following the recommendation of Littell 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 asrttests object

```
current.asr <- setvarianceterms(current.asr$call,
                                terms = c("Rep", "Rep:Row", "Rep:Column"),
                                bounds = "U")
```

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

```
current.asrt <- as.asrttests(current.asr, wald.tab = NULL, test.summary = current.asrt$test.summary,
                             IClikelihood = "full", label = "Max model & Unbound components")
current.asrt <- rmboundary(current.asrt)
summary(current.asrt$asreml.obj)$varcomp
```

	component	std.error	z.ratio	bound	%ch
Rep	-2458.3485841	1.197491e+03	-2.0529167	U	0.0
Rep:Row	5008.7151486	3.401335e+03	1.4725732	U	0.0
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
1	Maximal model	26	6	NA	1646.129	1742.469	Starting model
2	Rep	1	NA	NA	1646.129	1742.469	Boundary
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.asrttests`, 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 `asrttests` object.

```
current.asrt <- testtranfix(current.asrt, term = "WithinColPairs",
                           drop.fix.ns=TRUE, IClkelihood = "full")
```

Warning in asreml(fixed = yield ~ Variety, random = ~Rep/(Row + Column) + : 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

	component	std.error	z.ratio	bound	%ch
Rep	-2391.9489939	1.194581e+03	-2.0023338	U	0.4
Rep:Row	5035.5311054	3.406006e+03	1.4784269	U	0.3
Rep:Column	761.9535622	1.612103e+03	0.4726458	U	1.2
units	5933.2133794	1.610805e+03	3.6833848	P	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!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	p	AIC	BIC	action
1	Maximal model	26	6.0	NA	1646.129	1742.469	Starting model
2	Rep	1	NA	NA	1646.129	1742.469	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

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")
```

```
Warning in asreml(fixed = yield ~ Variety, random = ~Rep + Rep:Row + Rep:Column, : 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.

```
current.asrt <- testresidual(current.asrt, "~ Row:ar1(Column)",
                             label="Row autocorrelation",
                             simplifier=TRUE, IClikelihood = "full")
```

```
Warning in asreml(fixed = yield ~ Variety, random = ~Rep/(Row + Column) + : 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
```

```
current.asrt <- iterate(current.asrt)
```

## 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 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"))
```

```
[1] 4.676754e-06
```

```
{ if (p <= 0.05)
  current.asrt <- testresidual(current.asrt, "~ ar1(Row):Column",
                              label="Col autocorrelation",
                              simplifier=TRUE, IClikelihood = "full")
else
  current.asrt <- testresidual(current.asrt, "~ Row:Column",
                              label="Col autocorrelation",
                              simplifier=TRUE, IClikelihood = "full")
}
```

```
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	denDF	p	AIC	BIC	action
1	Maximal model	26	6.0	NA	1646.129	1742.469	Starting model
2	Rep	1	NA	NA	1646.129	1742.469	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 = ~ .))
```

```
[1] 44.62413
attr(,"fixed")
~.
<environment: 0x0000020ac21ae668>
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

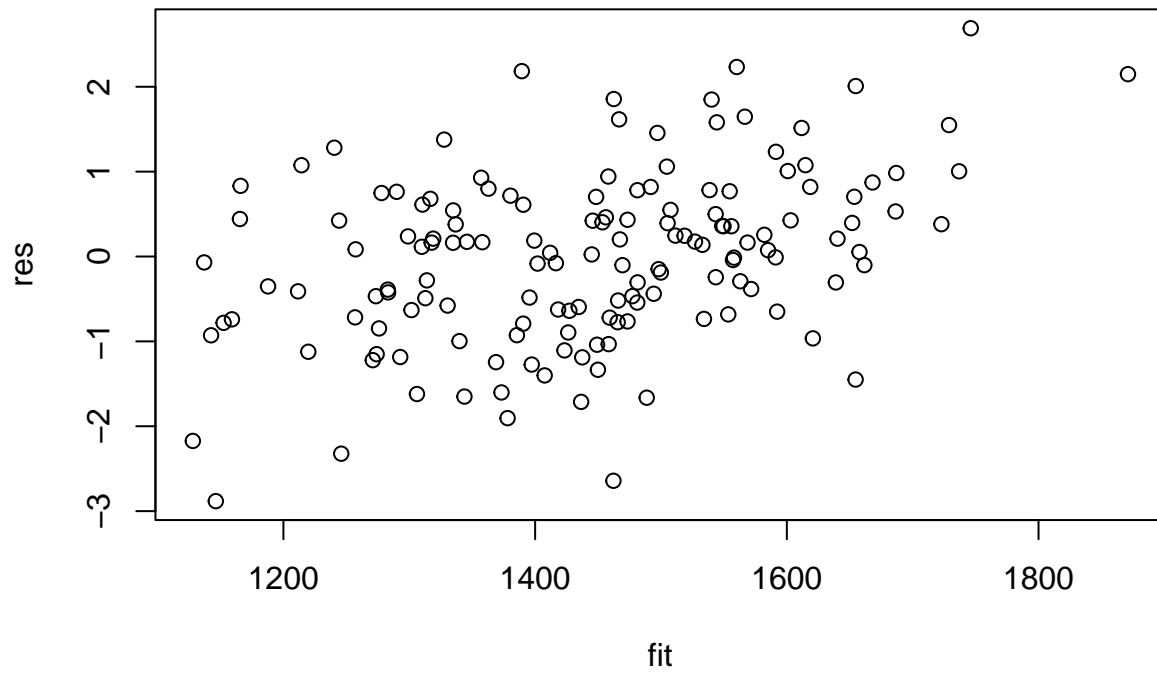
```
current.asr <- current.asrt$asreml.obj
current.asr <- update(current.asr, aom=TRUE)
Wheat.dat$res <- residuals(current.asr, type = "stdCond")
Wheat.dat$fit <- fitted(current.asr)
```

Do diagnostic checking

Do residuals-versus-fitted values plot



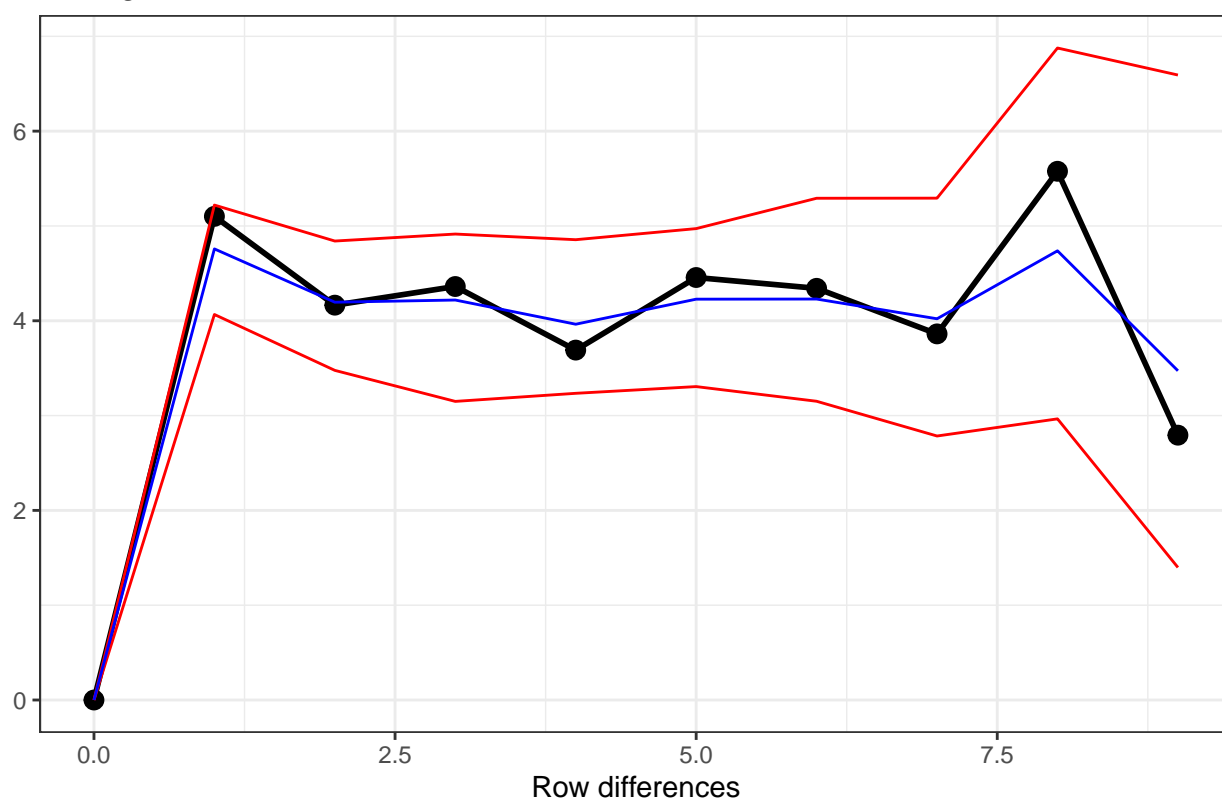
```
with(Wheat.dat, plot(fit, res))
```



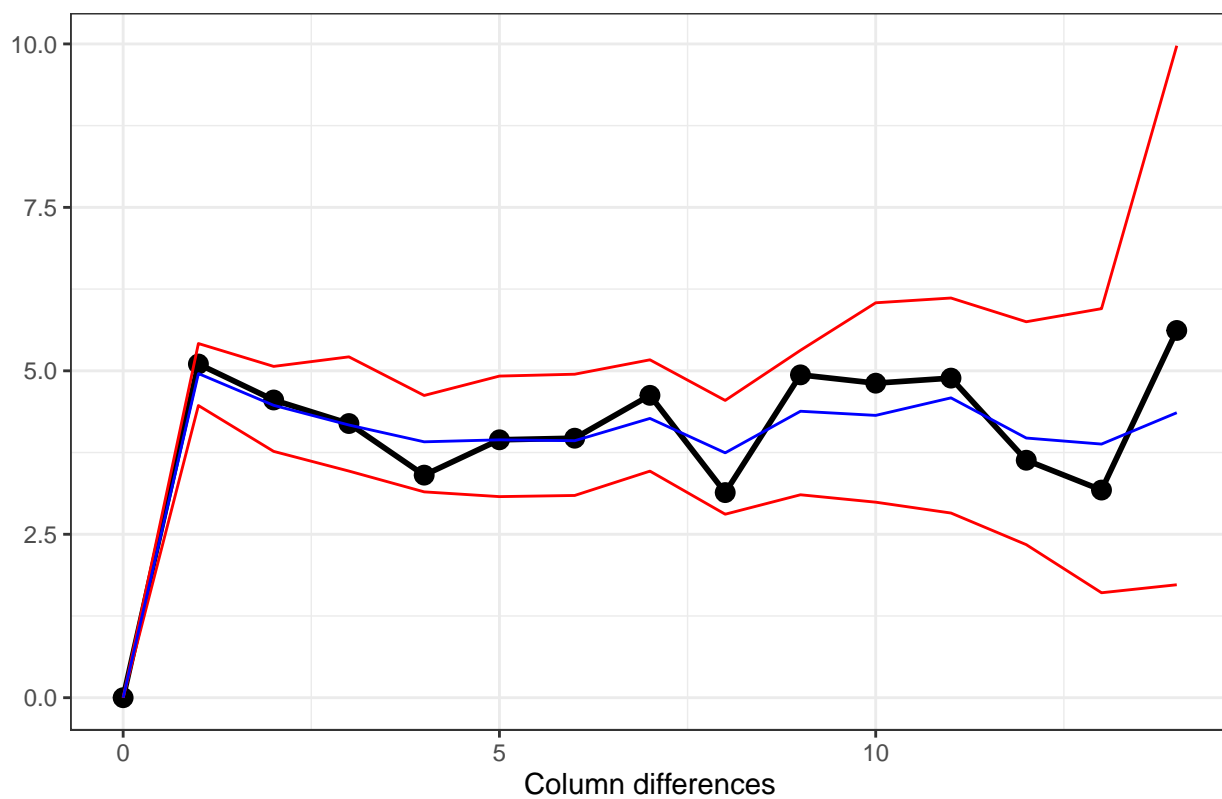
Plot variofaces

```
variofaces(current.asr, V=NULL, units="addtores",  
            maxiter=50, update = FALSE,  
            ncores = parallel::detectCores())
```

Variogram face of Standardized conditional residuals for Row



Variogram face of Standardized conditional residuals for Column



The variofaces are the lag 1 plots of the sample semivariogram with simulated confidence envelopes (Stefanova

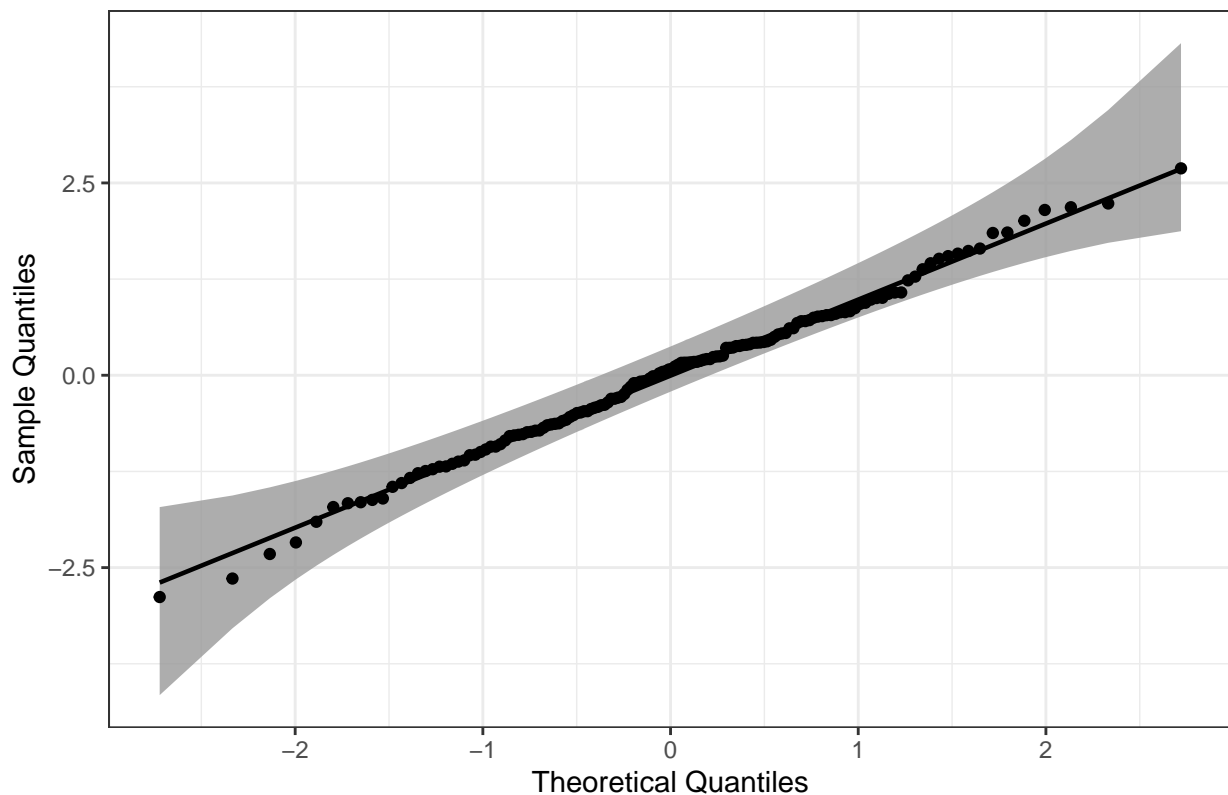
et al., 2009).

### 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())
```

Normal probability plot



## 4. Prediction production and presentation

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

```
Var.diffs <- predictPlus(classify = "Variety",  
  asreml.obj=current.asr,  
  error.intervals="halfLeast",  
  wald.tab=current.asrt$wald.tab,
```

```
sortFactor = "Variety",
tables = "predictions")
```

#### 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

	Variety	predicted.value	standard.error	upper.halfLeastSignificant.limit
1	10	1168.989	120.4768	1228.315
2	1	1242.750	119.8104	1302.076
3	9	1257.137	119.9708	1316.463
4	16	1285.718	119.9400	1345.045
5	14	1293.526	119.9227	1352.853
6	23	1313.653	120.2929	1372.979
7	11	1322.159	120.1964	1381.485
8	7	1374.447	120.2407	1433.773
9	3	1394.070	120.4032	1453.396
10	4	1410.980	120.1055	1470.306
11	12	1444.557	120.6034	1503.883
12	8	1453.396	120.5940	1512.723
13	15	1458.383	120.4346	1517.709
14	5	1473.782	120.4455	1533.108
15	17	1487.828	120.2896	1547.154
16	6	1498.294	120.1189	1557.620
17	21	1517.121	120.2262	1576.447
18	2	1520.466	119.6322	1579.792
19	24	1533.769	120.2995	1593.095
20	18	1541.148	120.3664	1600.474
21	25	1575.795	120.5142	1635.121
22	22	1610.482	120.3281	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.halfLeastSignificant.limit est.status			
1		1109.663	Estimable	
2		1183.424	Estimable	
3		1197.811	Estimable	
4		1226.392	Estimable	
5		1234.200	Estimable	
6		1254.327	Estimable	
7		1262.832	Estimable	
8		1315.120	Estimable	

9	1334.743	Estimable
10	1351.653	Estimable
11	1385.231	Estimable
12	1394.070	Estimable
13	1399.057	Estimable
14	1414.456	Estimable
15	1428.501	Estimable
16	1438.968	Estimable
17	1457.795	Estimable
18	1461.140	Estimable
19	1474.443	Estimable
20	1481.821	Estimable
21	1516.468	Estimable
22	1551.156	Estimable
23	1551.436	Estimable
24	1568.645	Estimable
25	1593.666	Estimable

LSD values

minimum LSD = 114.0128

mean LSD = 118.6523

maximum LSD = 123.3578

(sed range / mean sed = 0.0788 )

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).

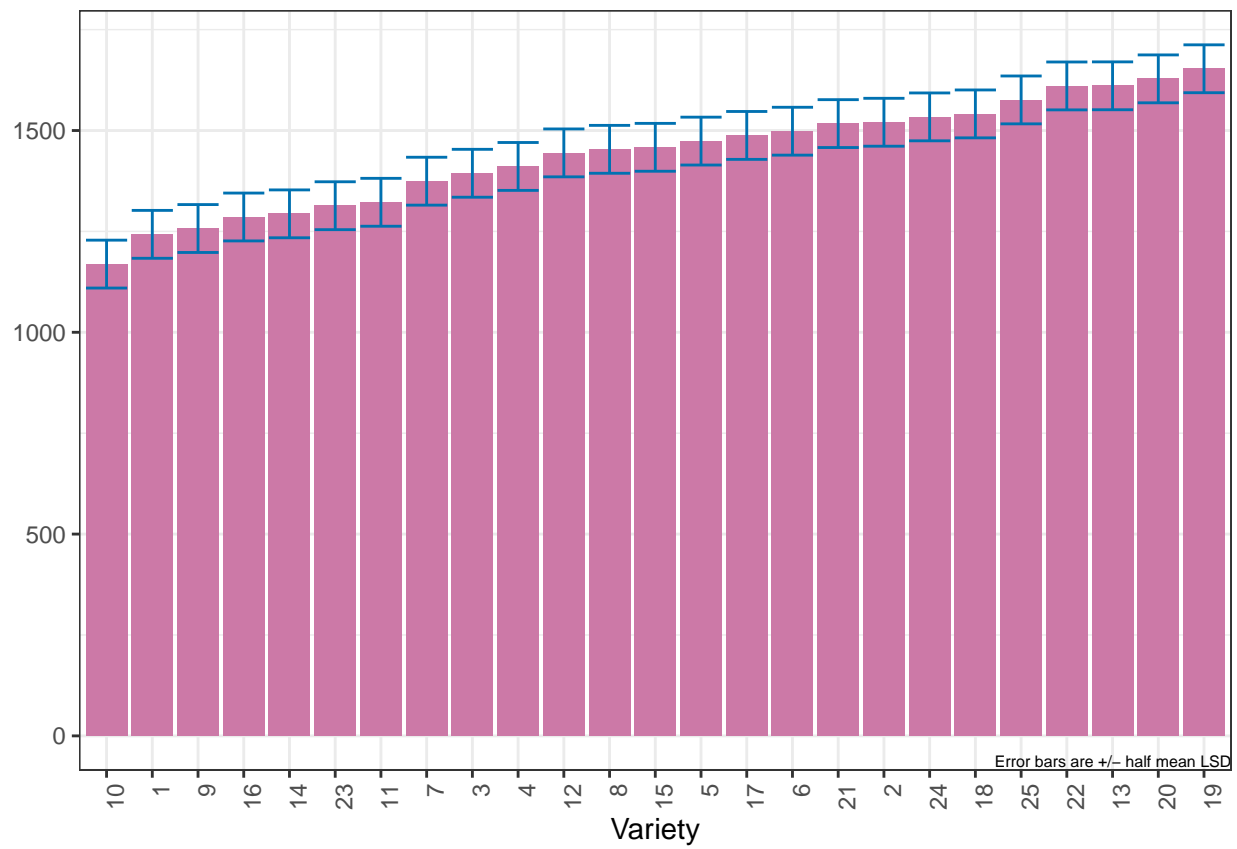
Also set was `sortFactor`, so that the results would be ordered for the values of the predictions for Variety.

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

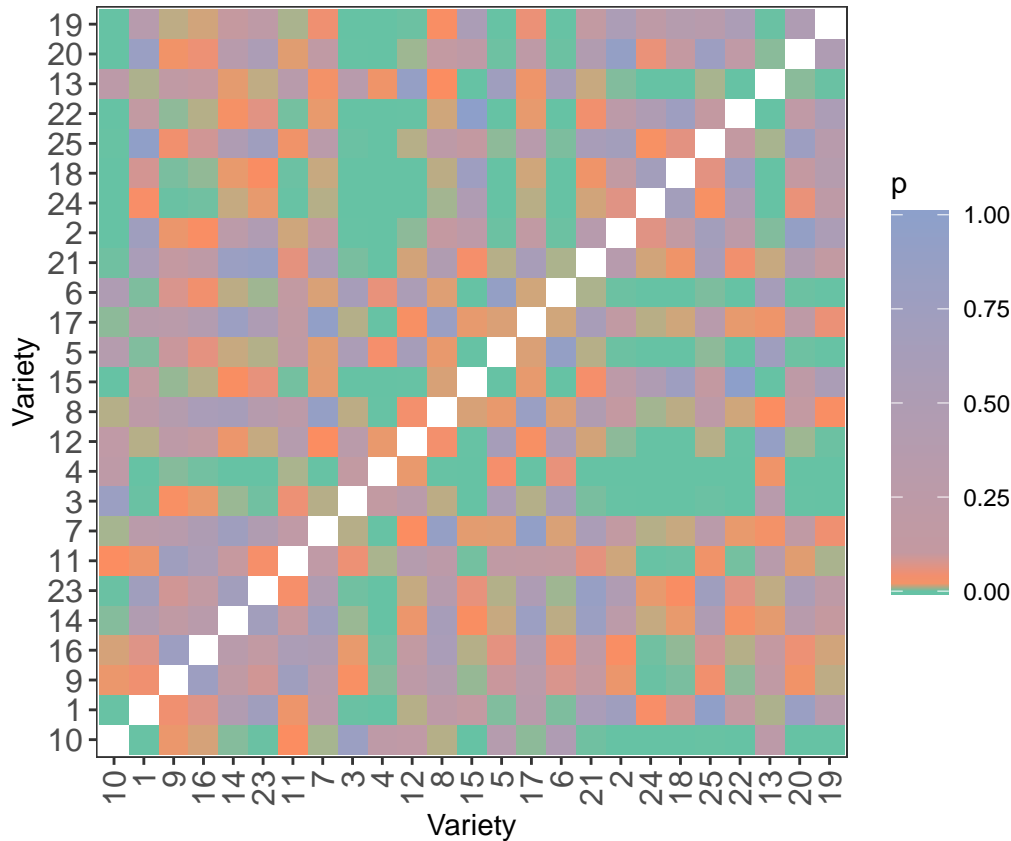
- `predictions`: the predictions, their standard errors and error intervals;
- `vcov`: the variance matrix of the predictions;
- `differences`: all pairwise differences between the predictions,
- `p.differences`: p-values for all pairwise differences between the predictions;
- `sed`: the standard errors 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**

```
plotPredictions(Var.diffs$predictions,
  classify = "Variety", y = "predicted.value",
  error.intervals = "half")
```



```
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 4.4.29. <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.vsnr.co.uk/>.
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- 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.
- R Core Team (2024) *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <https://www.r-project.org/>.
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- Stefanova, K. T., Smith, A. B. & Cullis, B. R. (2009) Enhanced diagnostics for the spatial analysis of field trials. *Journal of Agricultural, Biological, and Environmental Statistics*, **14**, 392–410.

Verbyla, A. P. (2019). A note on model selection using information criteria for general linear models estimated using REML. *Australian & New Zealand Journal of Statistics*, **61**, 39-50.<https://doi.org/10.1111/anzs.12254/>.