# 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, 2023), 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, 2023).

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 Thu Aug 24 10:19:11 2023
```

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

```
## [1] '4.2.0.267'
```

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

```
## [1] '4.4.13'
```

```
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),
                      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", IClikelihood = "full")
```

```
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")
```

```
Warning in infoCriteria.asreml(asreml.obj, IClikelihood = ic.lik): The following bound terms were disco
 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 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

```
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.asrtests(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.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.

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

```
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                   -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 1645.318 1741.658        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 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
```

## 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",
                             simpler=TRUE, IClikelihood = "full")
```

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

```
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 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] 4.676031e-06
```

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

```
####  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 1645.318 1741.658        Dropped
5                          units  1    NA 0.0006 1645.318 1741.658       Retained
6           Row autocorrelation  1    NA 0.0000 1645.318 1741.658      Unswapped
7           Col autocorrelation  2    NA 0.0000 1645.316 1741.656      Unswapped
```

```
printFormulae(current.asrt$asreml.obj)
```

```
#### Formulae from asreml object

fixed: yield ~ Variety
random: ~ Rep/(Row + Column) + units
residual: ~ ar1(Row):ar1(Column)
```

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

```
                         component    std.error    z.ratio bound %ch
Rep                   -2384.2946310 1.212190e+03 -1.9669310     U 0.0
Rep:Row                5026.4469057 3.417065e+03  1.4709837     U 0.0
Rep:Column              752.7496589 1.607683e+03  0.4682202     U 0.1
units                  5918.7214777 1.611779e+03  3.6721658     P 0.0
Row:Column!R          45854.0579175 2.620961e+04  1.7495130     P 0.0
Row:Column!Row!cor        0.8098355 1.002242e-01  8.0802373     U 0.0
```

```
Row:Column!Column!cor     0.8845749 7.513508e-02 11.7731282     U 0.0
```

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.

# 3. Diagnosing checking using residual plots and variofaces

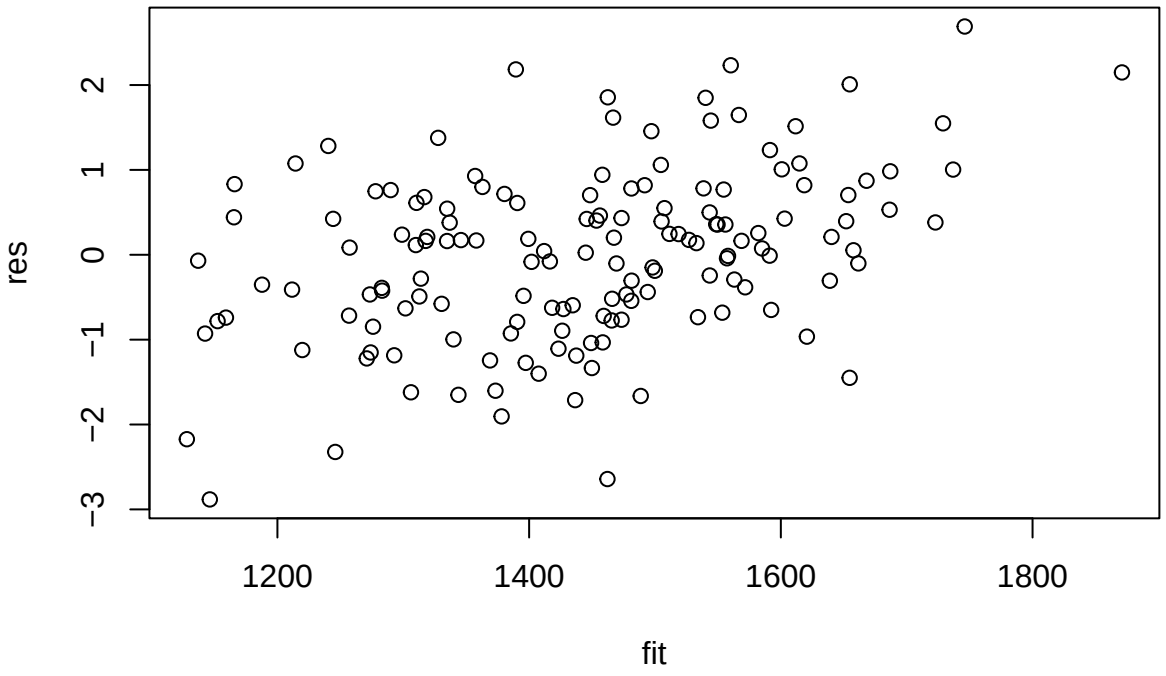
## Get current fitted asreml object and update to include standardized residuals

```
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

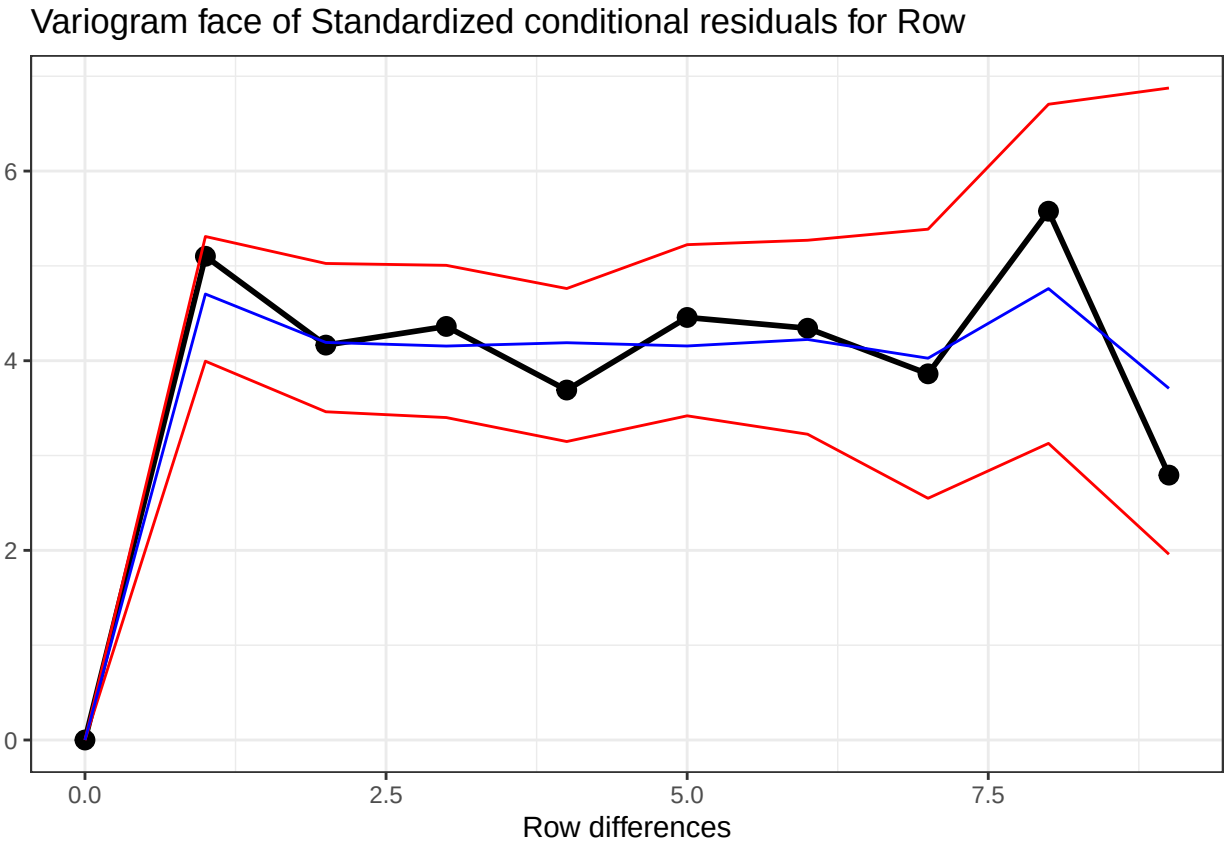
### Do residuals-versus-fitted values plot

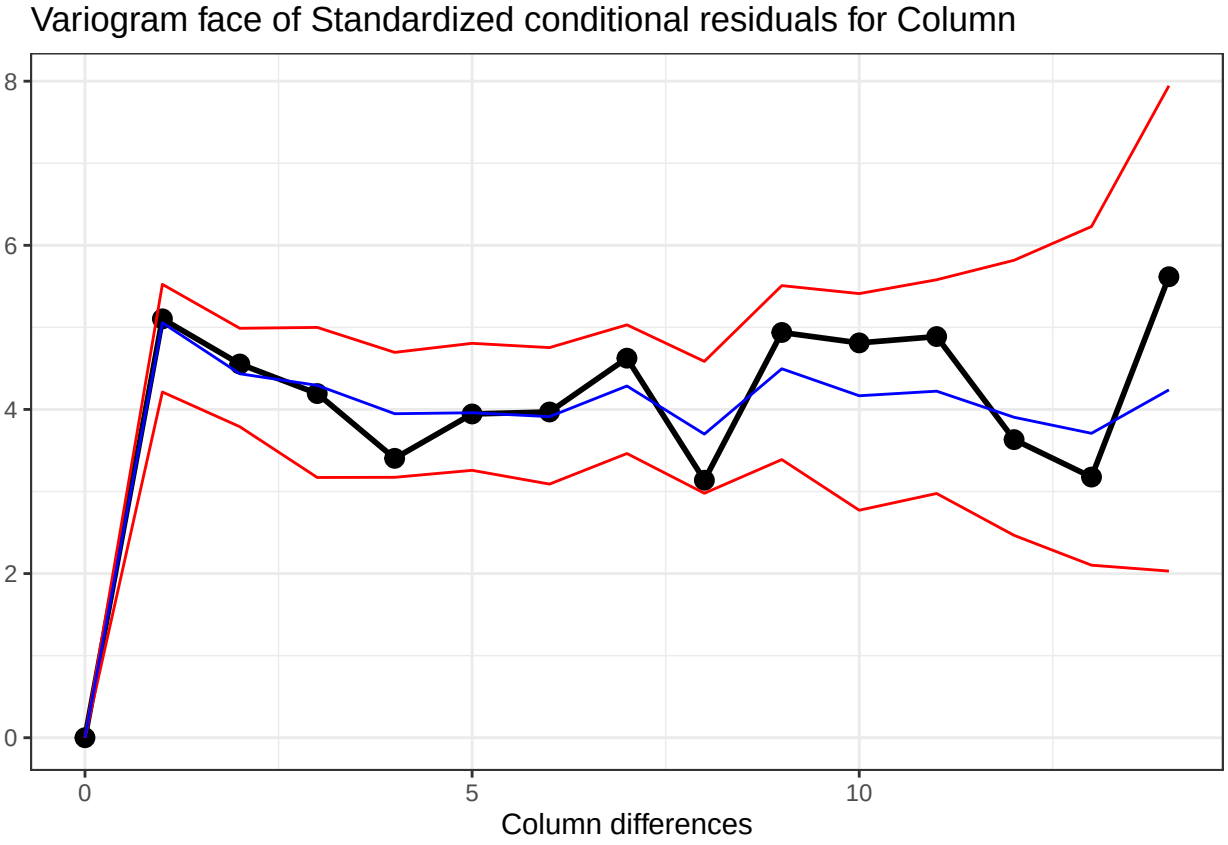
```
with(Wheat.dat, plot(fit, res))
```




### Plot variofaces

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


Variogram face of Standardized conditional residuals for Row
6
4
2
0
0.0
2.5
5.0
7.5
Row differences

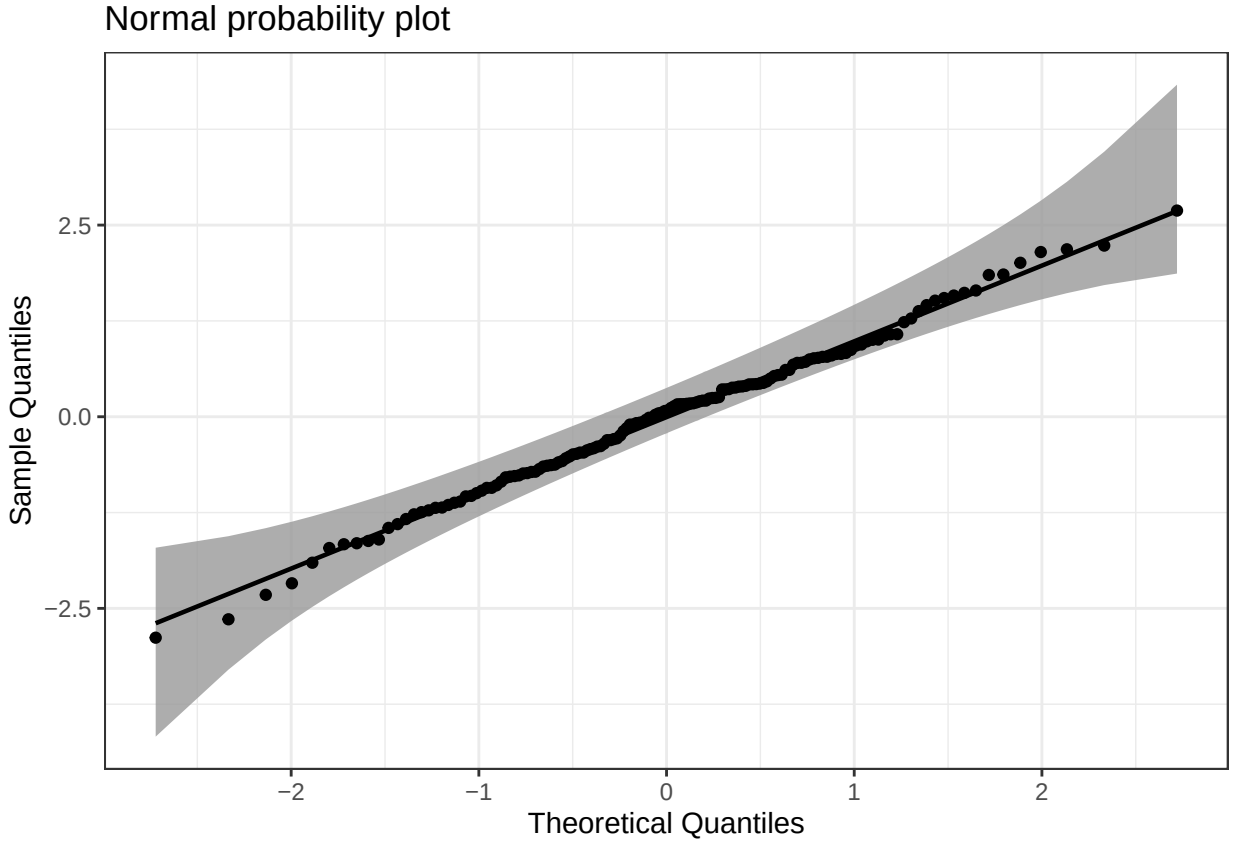

Variogram face of Standardized conditional residuals for Column
8
6
4
2
0
0
5
10
Column differences

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

```
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 | lower.halfLeastSignificant.limit | est.status |
|---|---|---|---|---|---|---|
| 1 | 10 | 1168.989 | 120.4766 | 1228.315 | 1109.663 | Estimable |
| 2 | 1 | 1242.750 | 119.8102 | 1302.076 | 1183.423 | Estimable |
| 3 | 9 | 1257.137 | 119.9706 | 1316.463 | 1197.811 | Estimable |
| 4 | 16 | 1285.718 | 119.9398 | 1345.045 | 1226.392 | Estimable |
| 5 | 14 | 1293.526 | 119.9225 | 1352.853 | 1234.200 | Estimable |
| 6 | 23 | 1313.653 | 120.2927 | 1372.979 | 1254.327 | Estimable |
| 7 | 11 | 1322.159 | 120.1962 | 1381.485 | 1262.833 | Estimable |
| 8 | 7 | 1374.447 | 120.2405 | 1433.773 | 1315.120 | Estimable |
| 9 | 3 | 1394.070 | 120.4030 | 1453.396 | 1334.744 | Estimable |
| 10 | 4 | 1410.980 | 120.1053 | 1470.306 | 1351.653 | Estimable |
| 11 | 12 | 1444.557 | 120.6033 | 1503.883 | 1385.231 | Estimable |
| 12 | 8 | 1453.397 | 120.5938 | 1512.723 | 1394.070 | Estimable |
| 13 | 15 | 1458.383 | 120.4344 | 1517.709 | 1399.057 | Estimable |
| 14 | 5 | 1473.782 | 120.4453 | 1533.108 | | |
| 15 | 17 | 1487.828 | 120.2894 | 1547.154 | | |
| 16 | 6 | 1498.294 | 120.1187 | 1557.620 | | |
| 17 | 21 | 1517.121 | 120.2260 | 1576.447 | | |
| 18 | 2 | 1520.466 | 119.6320 | 1579.792 | | |
| 19 | 24 | 1533.769 | 120.2993 | 1593.095 | | |
| 20 | 18 | 1541.147 | 120.3662 | 1600.474 | | |
| 21 | 25 | 1575.795 | 120.5140 | 1635.121 | | |
| 22 | 22 | 1610.482 | 120.3279 | 1669.808 | | |
| 23 | 13 | 1610.762 | 120.4573 | 1670.088 | | |
| 24 | 20 | 1627.971 | 120.2326 | 1687.297 | | |
| 25 | 19 | 1652.992 | 120.3433 | 1712.318 | | |

```
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).

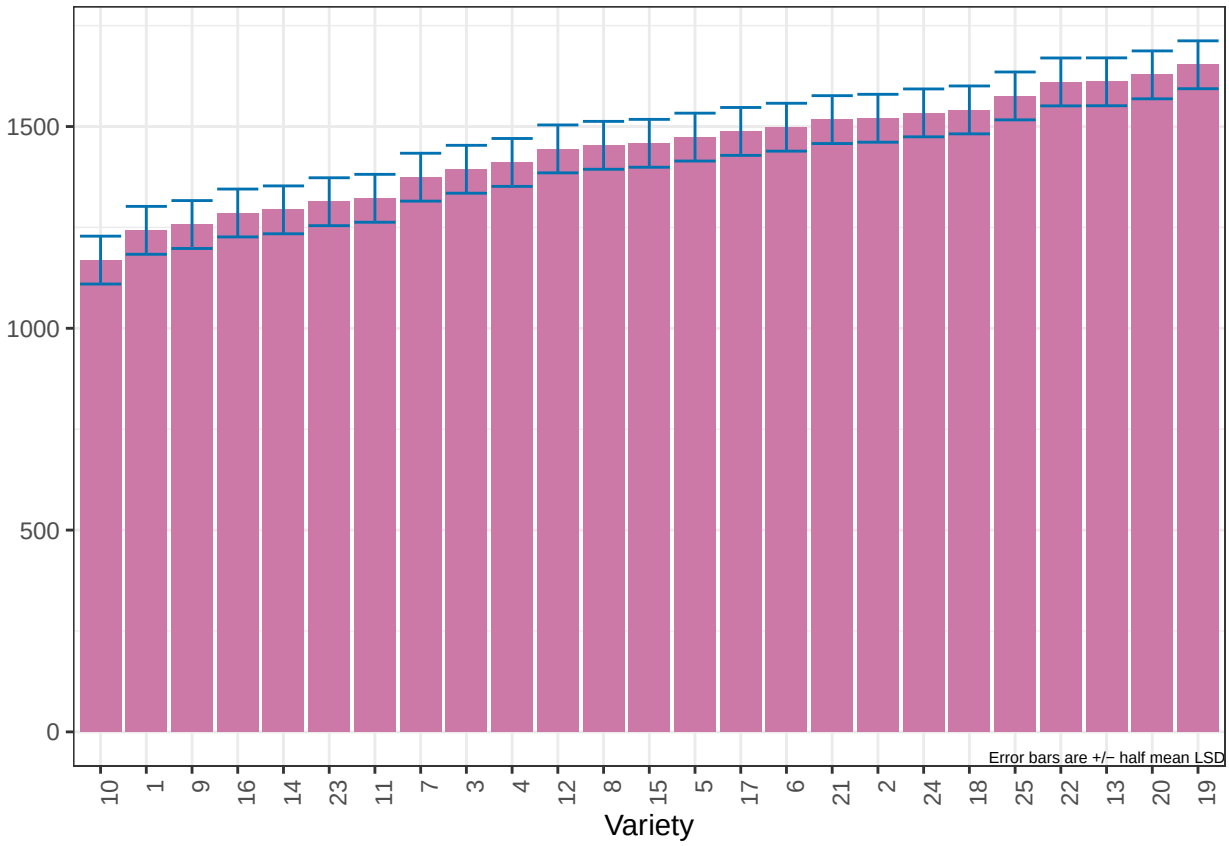
Also set was `sortFactor`, so that the results would be ordered for the values of the predictions for Variety.

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

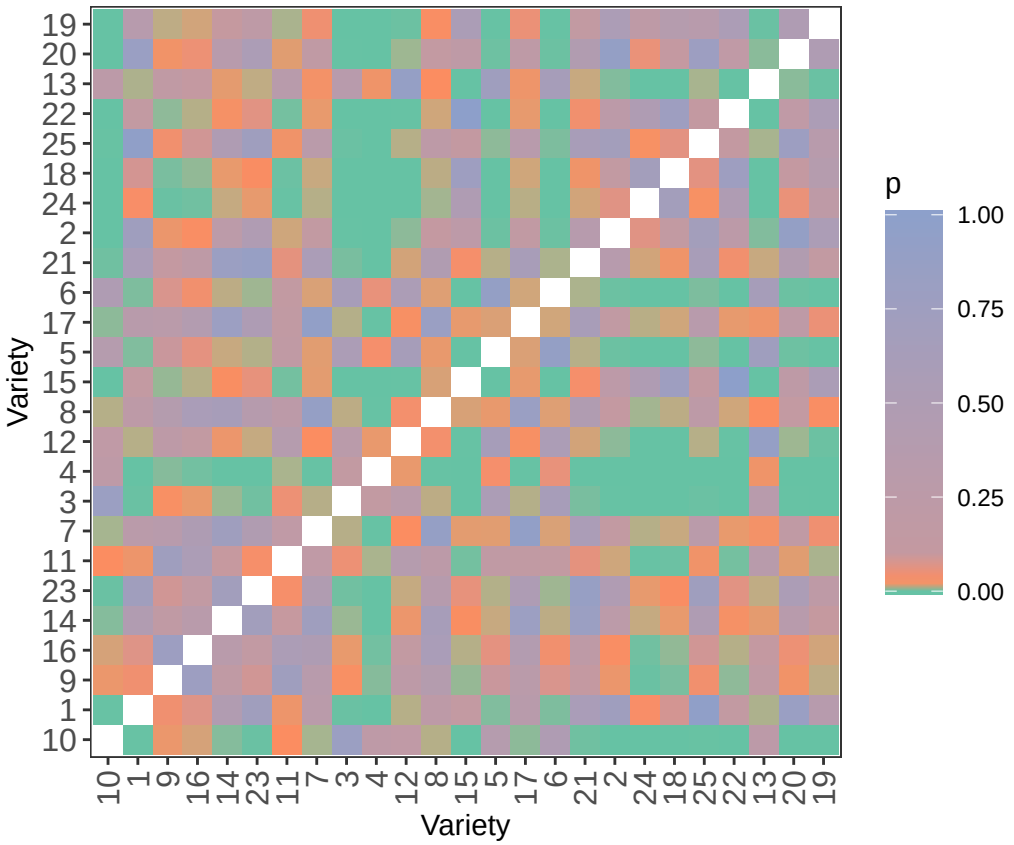
- `predictions`: the predictions, their standard errors and error intervals;
- `vcov`: the variance matrix of the predictions;
- `differences`: all pairwise differences between the predictions,
- `p.differences`: p-values for all pairwise differences between the predictions;
- `sed`: the standard errors 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. (2023) `asremlPlus`: *Augments ASReml-R in fitting mixed models and packages generally in exploring prediction differences.* Version 4.4.13. 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.

R Core Team (2023) `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/.