

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

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This vignette shows how to use **asremlPlus** (Brien, 2020), in conjunction with **asreml** (Butler et al., 2018), 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, 2020).

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))
packageVersion("asreml")
```

```
## [1] '4.1.0.110'
```

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

```
## [1] '4.2.12'
```

```
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. (2018, 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)
```

Model fitted using the gamma parameterization.

ASReml 4.1.0 Sun Feb 9 13:16:46 2020

	LogLik	Sigma2	DF	wall	cpu
1	-724.121	23034.14	124	13:16:46	0.0
2	-717.415	9206.93	124	13:16:46	0.0 (2 restrained)
3	-694.875	26492.99	124	13:16:46	0.0 (2 restrained)
4	-694.160	33101.80	124	13:16:46	0.0 (1 restrained)
5	-692.002	36912.26	124	13:16:46	0.0 (1 restrained)
6	-691.789	46701.51	124	13:16:46	0.0 (2 restrained)
7	-691.834	46208.51	124	13:16:46	0.0 (1 restrained)
8	-691.775	47698.26	124	13:16:46	0.0
9	-691.771	47041.85	124	13:16:46	0.0

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

```
current.asrt <- as.asrtests(current.asr, NULL, NULL)
```

Calculating denominator DF

Check for and remove any boundary terms

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

(For rows with AIC and BIC, DF and denDF relate to fixed and variance parameter numbers)

```
terms DF denDF p AIC BIC action
1 Rep 1 NA NA NA NA 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 `asrtests` object

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

Model fitted using the gamma parameterization.

ASReml 4.1.0 Sun Feb 9 13:16:47 2020

	LogLik	Sigma2	DF	wall	cpu
1	-724.121	23034.14	124	13:16:47	0.0
2	-717.415	9206.93	124	13:16:47	0.0 (2 restrained)
3	-694.875	26492.99	124	13:16:47	0.0 (2 restrained)
4	-693.974	33129.65	124	13:16:47	0.0 (1 restrained)
5	-692.886	39662.12	124	13:16:47	0.0
6	-691.428	53103.83	124	13:16:47	0.0
7	-691.239	48092.17	124	13:16:47	0.0
8	-691.181	47278.94	124	13:16:47	0.0
9	-691.171	46850.98	124	13:16:47	0.0
10	-691.170	46690.46	124	13:16:47	0.0

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, NULL, NULL)
```

Calculating denominator DF

```
current.asrt <- rmboundary(current.asrt)
summary(current.asrt$asreml.obj)$varcomp
```

	component	std.error	z.ratio	bound	%ch
Rep	-2462.3785859	1.191435e+03	-2.066734	U	0.2
Rep:Row	5012.4021416	3.396848e+03	1.475604	U	0.1
Rep:Column	920.5936392	1.704008e+03	0.540252	U	1.1
units	5964.9099379	1.608792e+03	3.707695	P	0.1
Row:Column!R	46690.4620402	2.731906e+04	1.709080	P	0.0
Row:Column!Row!cor	0.8152180	9.988929e-02	8.161216	U	0.1
Row:Column!Column!cor	0.8857252	7.487875e-02	11.828793	U	0.0

```
print(current.asrt, which = "testsummary")
```

Sequence of model investigations

(For rows with AIC and BIC, DF and denDF relate to fixed and variance parameter numbers)

```
[1] terms DF      denDF p      AIC    BIC    action
<0 rows> (or 0-length row.names)
```

```
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	153.400	0.0115
WithinColPairs	1	15.6	2.543	0.1308
Variety	24	76.1	10.110	0.0000

Now the Rep component estimate is negative.

The `test.summary` output shows that no changes have been made to the model loaded using `as.asrtests`. 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)

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

Calculating denominator DF

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

Calculating denominator DF

Generally, to determine what has been tested between two fits using `asreml` involves comparing two `asreml` calls and deciding what is different. For example what is the difference between the `asreml` call to fit the initial model and the following call?

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

On the other hand, it is clear from the `testranfix` call that the term `withinColPAirs` is being tested.

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)
```

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)
```

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 `newfit.asreml(asreml.obj, residual. = term.form, trace = trace, :`

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

Calculating denominator DF

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] 4.654398e-06
```

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

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
```

```
(For rows with AIC and BIC, DF and denDF relate to fixed and variance parameter numbers)
```

	terms	DF	denDF	p	AIC	BIC	action
1	WithinColPairs	1	15.6	0.1308	NA	NA	Dropped
2	units	1	NA	0.0006	NA	NA	Retained
3	Row autocorrelation	1	NA	0.0000	NA	NA	Unswapped - new unconverged
4	Col autocorrelation	2	NA	0.0000	NA	NA	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	-2385.9128545	1.211137e+03	-1.9699784	U	0.0
Rep:Row	5027.7499257	3.415346e+03	1.4721055	U	0.0
Rep:Column	753.6322682	1.609917e+03	0.4681187	U	0.6
units	5920.4104825	1.611261e+03	3.6743960	P	0.0
Row:Column!R	45870.5610760	2.623679e+04	1.7483295	P	0.0
Row:Column!Row!cor	0.8098804	1.001790e-01	8.0843355	U	0.0
Row:Column!Column!cor	0.8845772	7.510551e-02	11.7777939	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.asrttests. 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)
```

```

Model fitted using the gamma parameterization.
ASReml 4.1.0 Sun Feb  9 13:16:51 2020
      LogLik      Sigma2      DF      wall      cpu
1    -694.615    45855.43    125 13:16:51    0.0
2    -694.615    45854.15    125 13:16:51    0.0
3    -694.615    45851.11    125 13:16:51    0.0

```

```

Wheat.dat$res <- residuals(current.asr, type = "stdCond")
Wheat.dat$fit <- fitted(current.asr)

```

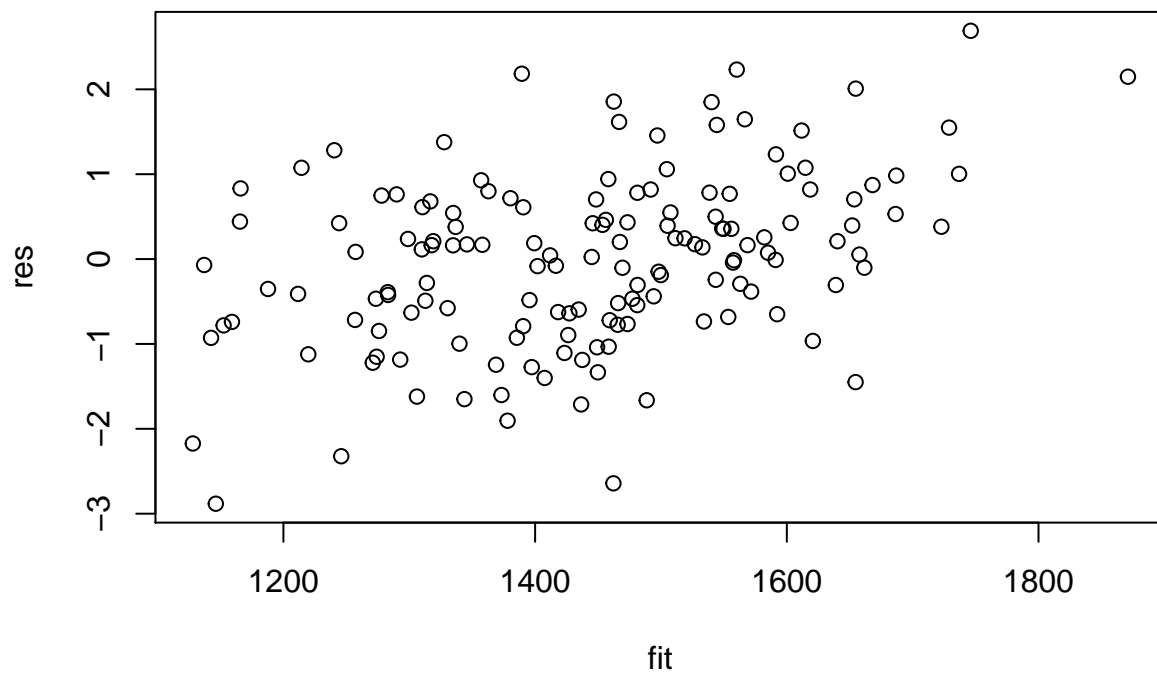
Do diagnostic checking

Do residuals-versus-fitted values plot

```

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

```



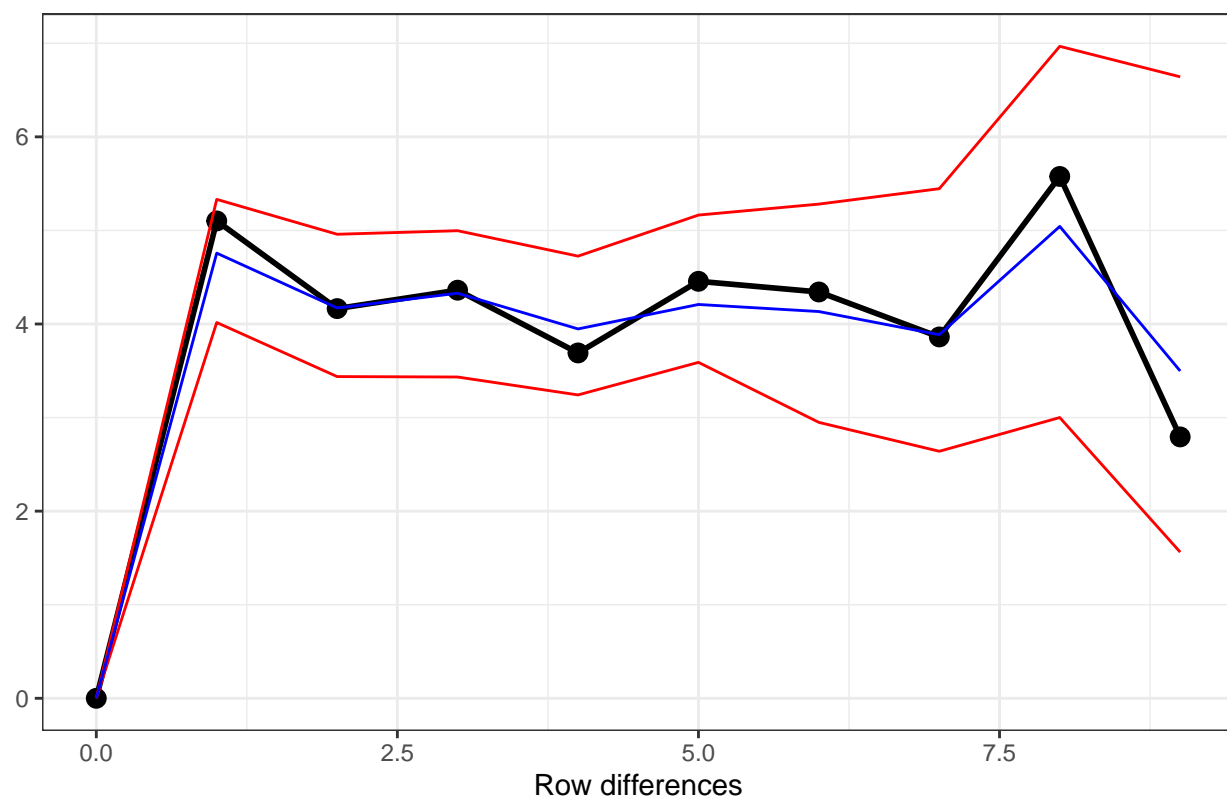
Plot variofaces

```

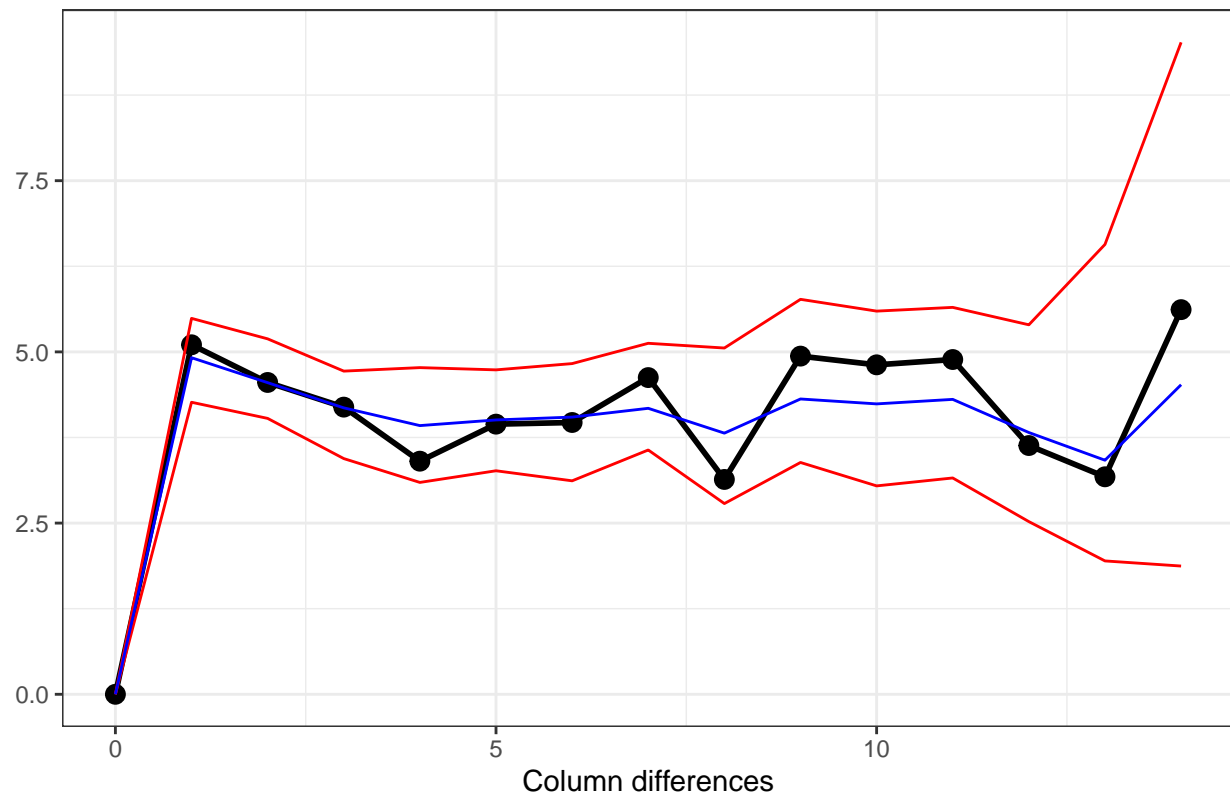
variofaces(current.asr, V=NULL, units="addtores",
            maxiter=50, update = FALSE)

```

Variogram face of Standardized conditional residuals for Row



Variogram face of Standardized conditional residuals for Column

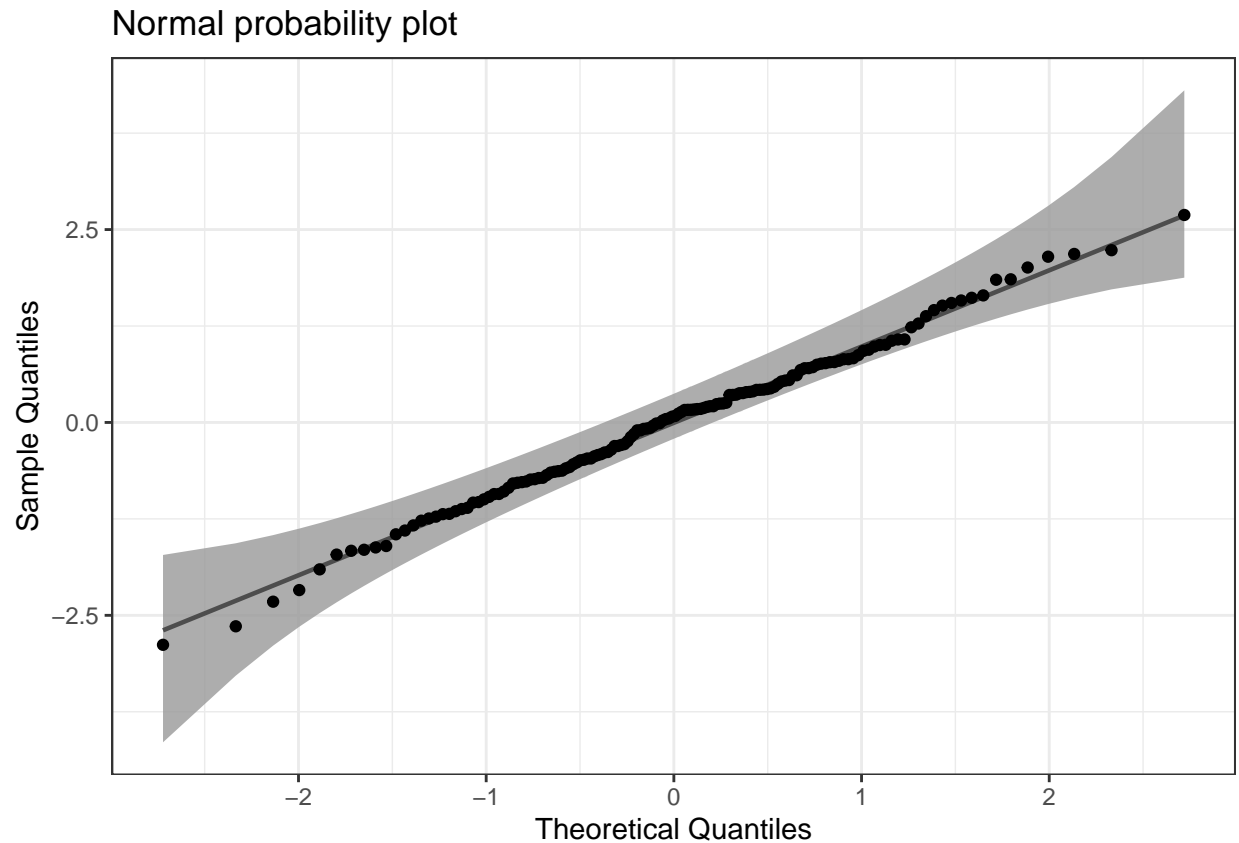


The variofaces are the lag 1 plots of the sample semivariogram with simulated confidence envelopes (Stefanova et al., 2009).

Plot normal quantile plot

The plot is obtained using the `ggplot` function with extensions available from the `qqplotr` package (Cheng, 2018).

```
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
10	10	1168.989	120.4768	1228.315
1	1	1242.750	119.8105	1302.076
9	9	1257.137	119.9708	1316.463
16	16	1285.718	119.9400	1345.045
14	14	1293.526	119.9227	1352.853
23	23	1313.653	120.2930	1372.979
11	11	1322.159	120.1964	1381.485
7	7	1374.447	120.2407	1433.773
3	3	1394.070	120.4032	1453.396
4	4	1410.980	120.1055	1470.306
12	12	1444.557	120.6034	1503.883
8	8	1453.396	120.5940	1512.723
15	15	1458.383	120.4346	1517.709
5	5	1473.782	120.4455	1533.108
17	17	1487.828	120.2896	1547.154
6	6	1498.294	120.1189	1557.620
21	21	1517.121	120.2262	1576.447
2	2	1520.466	119.6322	1579.792
24	24	1533.769	120.2995	1593.095
18	18	1541.148	120.3664	1600.474
25	25	1575.795	120.5142	1635.121
22	22	1610.482	120.3281	1669.808
13	13	1610.762	120.4575	1670.088
20	20	1627.971	120.2328	1687.297
19	19	1652.992	120.3435	1712.318
		lower.halfLeastSignificant.limit	est.status	
10		1109.663	Estimable	
1		1183.424	Estimable	
9		1197.811	Estimable	
16		1226.392	Estimable	
14		1234.200	Estimable	
23		1254.327	Estimable	
11		1262.832	Estimable	
7		1315.120	Estimable	
3		1334.743	Estimable	
4		1351.653	Estimable	
12		1385.231	Estimable	
8		1394.070	Estimable	
15		1399.057	Estimable	
5		1414.456	Estimable	
17		1428.501	Estimable	
6		1438.968	Estimable	
21		1457.795	Estimable	
2		1461.140	Estimable	
24		1474.443	Estimable	
18		1481.821	Estimable	
25		1516.468	Estimable	
22		1551.156	Estimable	
13		1551.436	Estimable	
20		1568.645	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 $\pm 0.5LSD$ 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).

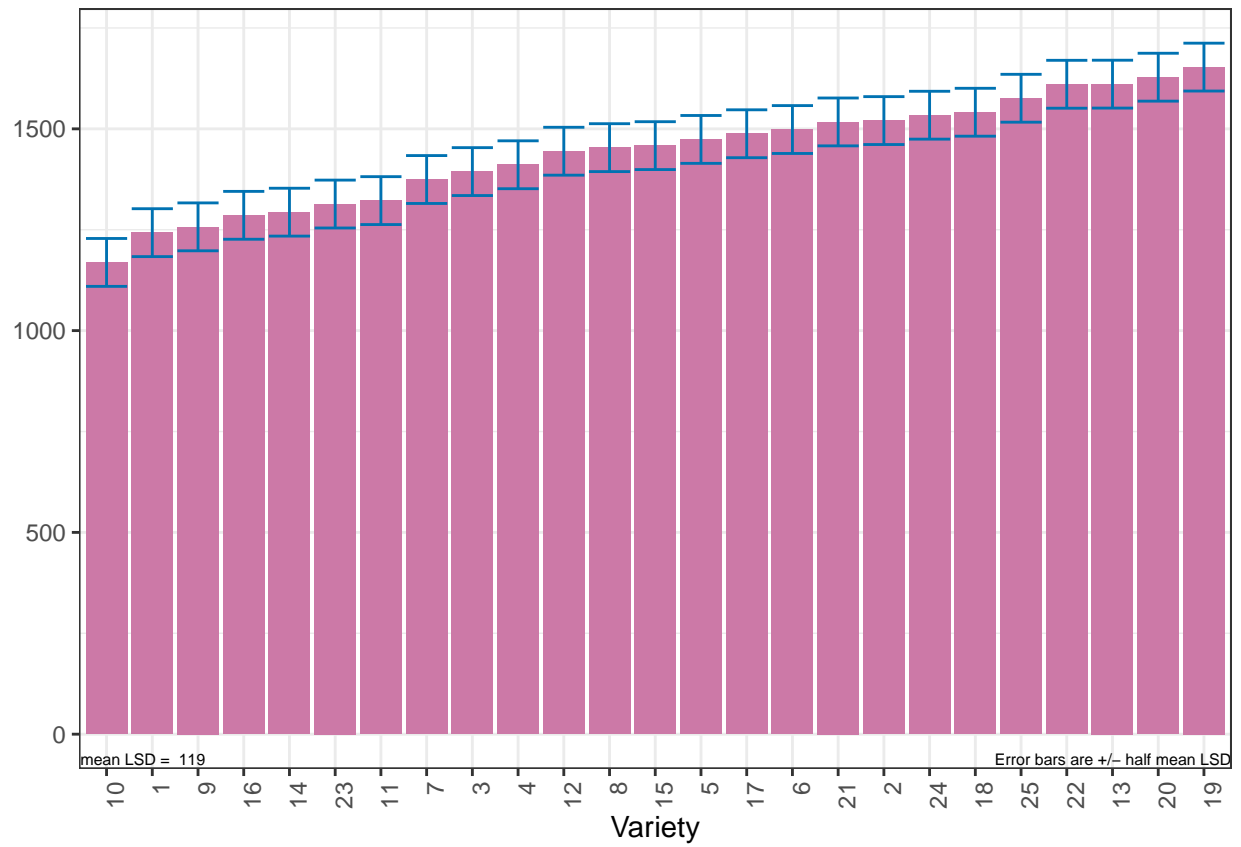
Also set was `sortFactor`, so that the results would be ordered for the values of the predictions for Variety.

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

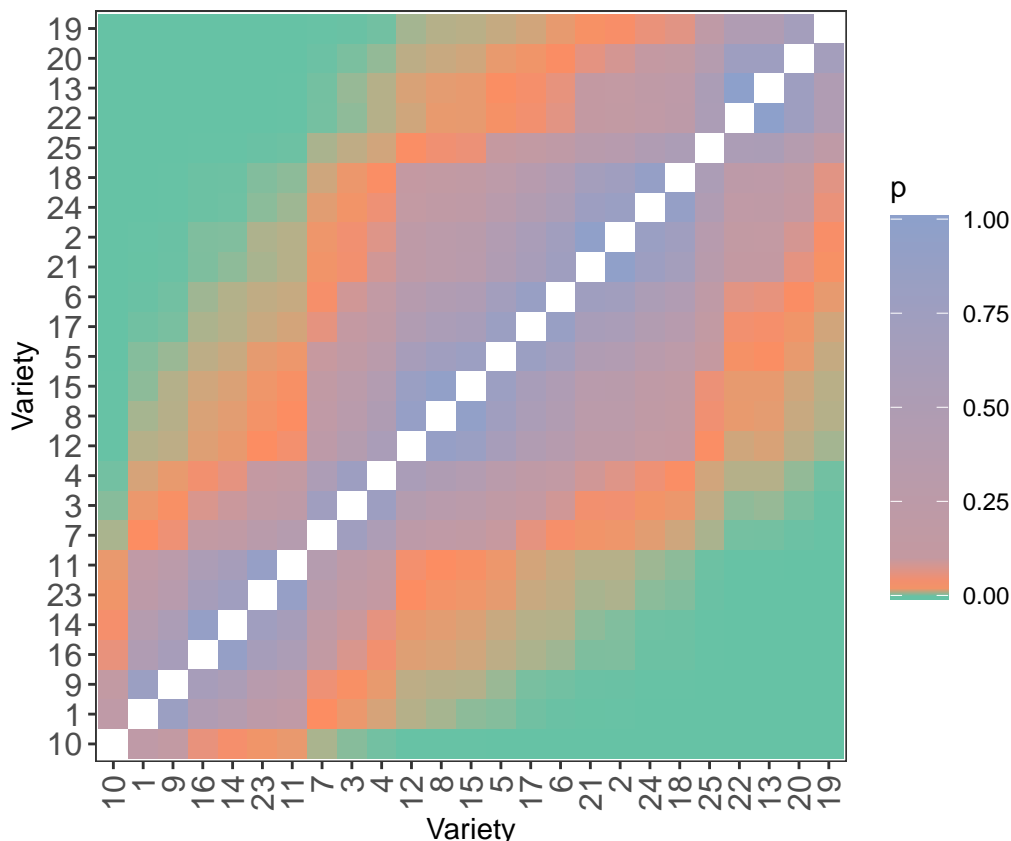
- `predictions`: the predictions, their standard errors and error intervals;
- `vcov`: the variance matrix of the predictions;
- `differences`: all pairwise differences between the predictions,
- `p.differences`: p-values for all pairwise differences between the predictions;
- `sed`: the standard errors 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 Heike Hofmann, H. (2017) *qqplotr: Quantile-Quantile plot extensions for 'ggplot2'*, Version 0.0.3. <http://cran.r-project.org/package=qqplotr/> or <https://github.com/aloy/qqplotr>.
- Brien, C. J. (2020) *asremlPlus: Augments ASReml-R in fitting mixed models and packages generally in exploring prediction differences*. Version 4.2-12. <http://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. (2018). *ASReml-R Reference Manual Version 4*. VSN International Ltd, <http://asreml.org>.
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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 (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.
- 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.