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

```r
library(knitr)
opts_chunk$set(" tidy" = FALSE, comment = NA)
suppressMessages(library(asreml, quietly=TRUE))
packageVersion("asreml")
## [1] '4.1.0.122'
suppressMessages(library(asremlPlus))
packageVersion("asremlPlus")
## [1] '4.2.17'
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.

```r
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.

```r
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 Mon Mar 16 09:22:56 2020

LogLik  Sigma2  DF wall cpu
1   -724.121  23034.14  124 09:22:56  0.0
2   -717.415   9206.93  124 09:22:56  0.0 (2 restrained)
3   -694.875  26492.99  124 09:22:56  0.0 (2 restrained)
4   -694.160  33101.80  124 09:22:56  0.0 (1 restrained)
5   -692.002  36912.26  124 09:22:56  0.0 (1 restrained)
6   -691.789  46701.51  124 09:22:56  0.0 (2 restrained)
7   -691.834  46208.51  124 09:22:56  0.0 (1 restrained)
8   -691.775  47698.26  124 09:22:56  0.0
9   -691.771  47041.85  124 09:22:56  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**

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

```r
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.

Calculating denominator DF

**Check for and remove any boundary terms**

```r
current.asrt <- rmboundary(current.asrt, IClikelihood = "full")
```

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

```
Rep
```

Model fitted using the gamma parameterization.

```
ASReml 4.1.0 Mon Mar 16 09:22:57 2020

LogLik  Sigma2  DF wall cpu
1  -691.771  47071.42  124 09:22:57  0.0
```

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

```
summary(current.asrt$asreml.obj)$varcomp
```
#### Sequence of model investigations

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

<table>
<thead>
<tr>
<th>terms</th>
<th>DF</th>
<th>denDF</th>
<th>p</th>
<th>AIC</th>
<th>BIC</th>
<th>action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Maximal model</td>
<td>26</td>
<td>NA</td>
<td>1646.129</td>
<td>1742.469</td>
<td></td>
<td>Starting model</td>
</tr>
<tr>
<td>2 Rep</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>1646.129</td>
<td>1742.469</td>
<td>Boundary</td>
</tr>
</tbody>
</table>

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

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

Model fitted using the gamma parameterization.

ASReml 4.1.0 Mon Mar 16 09:22:58 2020

<table>
<thead>
<tr>
<th>LogLik</th>
<th>Sigma2</th>
<th>DF</th>
<th>wall</th>
<th>cpu</th>
<th>wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-724.121</td>
<td>23034.14</td>
<td>124</td>
<td>09:22:58</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>-717.415</td>
<td>9206.93</td>
<td>124</td>
<td>09:22:58</td>
<td>0.0 (2 restrained)</td>
</tr>
<tr>
<td>3</td>
<td>-694.875</td>
<td>26492.99</td>
<td>124</td>
<td>09:22:58</td>
<td>0.0 (2 restrained)</td>
</tr>
<tr>
<td>4</td>
<td>-693.974</td>
<td>33129.65</td>
<td>124</td>
<td>09:22:58</td>
<td>0.0 (1 restrained)</td>
</tr>
<tr>
<td>5</td>
<td>-692.886</td>
<td>39662.12</td>
<td>124</td>
<td>09:22:58</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>-691.428</td>
<td>53103.83</td>
<td>124</td>
<td>09:22:58</td>
<td>0.0</td>
</tr>
<tr>
<td>7</td>
<td>-691.239</td>
<td>48092.17</td>
<td>124</td>
<td>09:22:58</td>
<td>0.0</td>
</tr>
<tr>
<td>8</td>
<td>-691.181</td>
<td>47278.94</td>
<td>124</td>
<td>09:22:58</td>
<td>0.0</td>
</tr>
<tr>
<td>9</td>
<td>-691.171</td>
<td>46850.98</td>
<td>124</td>
<td>09:22:58</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>-691.170</td>
<td>46690.46</td>
<td>124</td>
<td>09:22:58</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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

```r
current.asr <- as.asrtests(current.asr, wald.tab = NULL, test.summary = current.asr$test.summary, IClikelihood = "full", label = "Max model & Unbound components")
```

Calculating denominator DF

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

<table>
<thead>
<tr>
<th>component</th>
<th>std.error</th>
<th>z.ratio</th>
<th>bound</th>
<th>%ch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep:Row</td>
<td>3.199458e+03</td>
<td>1.3418779</td>
<td>P</td>
<td>0.0</td>
</tr>
<tr>
<td>Rep:Column</td>
<td>1.480357e+03</td>
<td>0.1064398</td>
<td>P</td>
<td>0.7</td>
</tr>
<tr>
<td>units</td>
<td>3.4752438</td>
<td>P</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Row:Column</td>
<td>1.8708669</td>
<td>P</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Row:Column!cor</td>
<td>7.8056280</td>
<td>U</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Row:Column!Column!cor</td>
<td>11.9390486</td>
<td>U</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>
### Sequence of model investigations

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

<table>
<thead>
<tr>
<th>terms</th>
<th>DF</th>
<th>denDF</th>
<th>p</th>
<th>AIC</th>
<th>BIC</th>
<th>action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal model</td>
<td>26</td>
<td>6</td>
<td>NA</td>
<td>1646.129</td>
<td>1742.469</td>
<td>Starting model</td>
</tr>
<tr>
<td>Rep</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>1646.129</td>
<td>1742.469</td>
<td>Boundary</td>
</tr>
<tr>
<td>Max model &amp; Unbound components</td>
<td>26</td>
<td>7</td>
<td>NA</td>
<td>1647.200</td>
<td>1746.551</td>
<td>Starting model</td>
</tr>
</tbody>
</table>

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 ($p < 0.001$)

### Pseudo-anova table for fixed terms

Wald tests for fixed effects.
Response: yield

<table>
<thead>
<tr>
<th>Df denDF</th>
<th>F.inc</th>
<th>Pr</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept) 1</td>
<td>153.400</td>
<td>0.0115</td>
</tr>
<tr>
<td>WithinColPairs 1</td>
<td>2.543</td>
<td>0.1308</td>
</tr>
<tr>
<td>Variety 24</td>
<td>10.110</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

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.

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

4
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?

```r
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.

```r
current.asrt <- testranfix(current.asr, "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.

```r
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 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 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", 
  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)

<table>
<thead>
<tr>
<th>terms</th>
<th>DF</th>
<th>denDF</th>
<th>p</th>
<th>AIC</th>
<th>BIC</th>
<th>action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Maximal model 26 6.0 NA 1646.129 1742.469</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Starting model</td>
</tr>
<tr>
<td>2 Rep 1 NA NA 1646.129 1742.469</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Boundary</td>
</tr>
<tr>
<td>3 Max model &amp; Unbound components 26 7.0 NA 1647.200 1746.551</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Starting model</td>
</tr>
<tr>
<td>4 WithinColPairs 1 15.6 0.1308 1645.326 1741.666</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dropped</td>
</tr>
<tr>
<td>5 units 1 NA 0.0006 1645.326 1741.666</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Retained</td>
</tr>
<tr>
<td>6 Row autocorrelation 1 NA 0.0000 1645.326 1741.666 Unswapped - new unconverged</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Col autocorrelation 2 NA 0.0000 1645.318 1741.658</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Unswapped</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>component</th>
<th>std.error</th>
<th>z.ratio</th>
<th>bound</th>
<th>%ch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep</td>
<td>-2385.9128545</td>
<td>1.211137e+03</td>
<td>-1.9699784</td>
<td>U 0.0</td>
</tr>
<tr>
<td>Rep:Row</td>
<td>5027.7499257</td>
<td>3.415346e+03</td>
<td>1.4721055</td>
<td>U 0.0</td>
</tr>
<tr>
<td>Rep:Column</td>
<td>753.6322682</td>
<td>1.609917e+03</td>
<td>0.4681187</td>
<td>U 0.6</td>
</tr>
<tr>
<td>units</td>
<td>5920.4104825</td>
<td>1.611261e+03</td>
<td>3.6743960</td>
<td>P 0.0</td>
</tr>
<tr>
<td>Row:Column!R</td>
<td>45870.5610760</td>
<td>2.623679e+04</td>
<td>1.7483295</td>
<td>P 0.0</td>
</tr>
<tr>
<td>Row:Column!Row!cor</td>
<td>0.8098804</td>
<td>1.001790e-01</td>
<td>8.0843355</td>
<td>U 0.0</td>
</tr>
</tbody>
</table>
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

```r
current.asr <- current.asrt$asreml.obj
current.asr <- update(current.asr, aom=TRUE)
```

Model fitted using the gamma parameterization.

```
   LogLik   Sigma2  DF  wall   cpu
1 -694.615  45855.43 125 09:23:03  0.0
2 -694.615  45854.15 125 09:23:03  0.0
3 -694.615  45851.11 125 09:23:03  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

```r
with(Wheat.dat, plot(fit, res))
```
Plot variofaces

```r
variofaces(current.asr, V=NULL, units="adtores",
           maxiter=50, update = FALSE)
```
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 (Cheng, 2018).

```r
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

```r
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
<table>
<thead>
<tr>
<th>Variety</th>
<th>Predicted Value</th>
<th>Standard Error</th>
<th>Upper Half Least Significant Limit</th>
<th>Lower Half Least Significant Limit</th>
<th>Est. Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1168.989</td>
<td>120.4768</td>
<td>1228.315</td>
<td>1109.663</td>
<td>Estimable</td>
</tr>
<tr>
<td>1</td>
<td>1242.750</td>
<td>119.8105</td>
<td>1302.076</td>
<td>1183.424</td>
<td>Estimable</td>
</tr>
<tr>
<td>9</td>
<td>1257.137</td>
<td>119.9708</td>
<td>1316.463</td>
<td>1197.811</td>
<td>Estimable</td>
</tr>
<tr>
<td>16</td>
<td>1285.718</td>
<td>119.9400</td>
<td>1345.045</td>
<td>1226.392</td>
<td>Estimable</td>
</tr>
<tr>
<td>14</td>
<td>1293.526</td>
<td>119.9227</td>
<td>1352.853</td>
<td>1234.200</td>
<td>Estimable</td>
</tr>
<tr>
<td>23</td>
<td>1313.653</td>
<td>120.2930</td>
<td>1372.979</td>
<td>1254.327</td>
<td>Estimable</td>
</tr>
<tr>
<td>11</td>
<td>1322.159</td>
<td>120.1964</td>
<td>1381.485</td>
<td>1262.832</td>
<td>Estimable</td>
</tr>
<tr>
<td>7</td>
<td>1374.447</td>
<td>120.2407</td>
<td>1433.773</td>
<td>1315.120</td>
<td>Estimable</td>
</tr>
<tr>
<td>3</td>
<td>1394.070</td>
<td>120.4032</td>
<td>1453.396</td>
<td>1334.743</td>
<td>Estimable</td>
</tr>
<tr>
<td>4</td>
<td>1410.980</td>
<td>120.1055</td>
<td>1470.306</td>
<td>1351.653</td>
<td>Estimable</td>
</tr>
<tr>
<td>12</td>
<td>1444.557</td>
<td>120.6034</td>
<td>1503.883</td>
<td>1385.231</td>
<td>Estimable</td>
</tr>
<tr>
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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 ±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 all diffs 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.diff$s$predictions, classify = "Variety", y = "predicted.value", error.intervals = "half")
Error bars are +/- half mean LSD. 

mean LSD = 119

Variety

plotPvalues(Var.diffs)
References


