IMPACT OF INCORRECT ASSUMPTIONS ON THE COVARIANCE STRUCTURE OF RANDOM EFFECTS AND/OR RESIDUALS IN NONLINEAR MIXED MODELS FOR REPEATED MEASURES DATA.

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Abstract

In this paper we analyse, using Monte Carlo simulation, the possible consequences of incorrect assumptions on the true structure of the random effects covariance matrix and the true correlation pattern of residuals, over the performance of an estimation method for nonlinear mixed models. The procedure under study is the well known linearization method due to Lindstrom and Bates (1990), implemented in the *nlme* library of S-Plus and R. Its performance is studied in terms of bias, mean square error (MSE), and true coverage of the associated asymptotic confidence intervals. Ignoring other criteria like the convenience of avoiding over parameterised models, it seems worst to erroneously assume some structure than do not assume any structure when this would be adequate.

Key Words: nlme, bias, confidence intervals, linearization, breast cancer, Soybean genotypes.

1 Introduction

Nonlinear mixed-effects (NLME) models are widely used tools for modelling and analysing repeated measures and hierarchical data. They allow the study of multiple sources of

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heterogeneity and/or correlation in data through the inclusion of random effects and explanative variables in the model.

The modelling process using mixed models is highly interactive and conducted in a step-by-step way, progressively incorporating hypotheses about the covariance structure, among other refinements. Then, it is reasonable to pose the question on the performance of these methods when the assumed covariance and/or correlation structures are inappropriate. This is a recurring question in the generalised linear models and generalised linear mixed models literature. Crowder (1995, 2001) points that the use of an incorrect correlation matrix could cause an inconsistency problem, when using the generalized estimating equations (GEE) for analysing repeated measures. His results are based on asymptotic theory. Sutradhar and Das (1999) obtain similar results and suggest a more robust estimation framework. Similarly, Chaganty (1997) introduces an alternative method in order to overcome these pitfalls of the GEE approach. On the other hand, the simulation results of Park and Yun Shin (1999) suggest that the GEE estimation method is robust under misspecifications of the correlation structure, even for moderate sample sizes. In this paper, we try to make some similar insights on the performance of a nonlinear mixed effects fitting procedure, the Lindstrom and Bates (1990) approach (from now LB method or approximation), that is widely used by practitioners.

A common approach to data analysis under the perspective of nonlinear mixed models consists in specifying the model and estimating their parameters using maximum likelihood or restricted maximum likelihood. A full likelihood analysis of NLMEs often requires difficult numerical integration or linear approximation methodology. The LB method is based on a Taylor-series expansion of the likelihood around successive approximations to the values of the fixed parameters and random effects. It consists in a two-step iterative process alternating between maximization of the log likelihood, given the above-mentioned values, and the generation of new parameter values using the linear mixed-effects methodology. The algorithm is based on the assumption that the intra-individual covariance matrix does not depend on the fixed parameters. This assumption can be justified by the fact that the matrix obtained by

differentiation of the objective function respect to the random effects, varies somewhat slowly with respect to fixed effects (Bates and Watts, 1980).

We present two simulation studies, each one consisting itself in two differentiated parts. In the first part of each simulation study, the consequences of fitting inappropriate restricted models were investigated. In the second part, the reverse case was investigated: the fitting process was performed under unrestricted conditions but data were generated according to some true structuring patterns. In the first simulation study, data were generated according to a model inspired in the breast cancer data analysed in El Halimi et al. (2003). In order to guess the general validity of the conclusions, the second simulation study was performed under a model based on the classical (in mixed models literature) Soybean genotypes dataset analysed, for example, in Pinheiro and Bates (2000) and in Davidian and Giltinan (2004).

In sections 2 and 3 we describe the design and the results of the first simulation study (breast cancer scenario) with some detail. The second simulation study (Soybean genotypes simulation scenario) is reported in section 4.

2 Simulation design

In the first series of simulations, the base model was:

$$y_{ij} = \frac{[\delta_1 + \eta_{1i}] \cdot \exp(t_{ij} - [\delta_2 + \eta_{2i}])}{1 + \exp\left(\frac{(t_{ij} - [\delta_2 + \eta_{2i}])}{\delta_3 + \eta_{3i}}\right)} + e_{ij} \qquad [1]$$

where the observations y_{ij} correspond to the simulated tumoural volume of subject i (i=1,...,m=38) at time t_{ij} (j=1,...,n_i=25), the random effects $\eta_i = (\eta_{1i}, \eta_{2i}, \eta_{3i})'$ are assumed to be normal with zero mean and covariance D and e_{ij} is a within-subject error, assumed normal with zero mean and variance σ^2. The model and the (pseudo-)data generation process are described with more detail in El Halimi et al.(2004).

In the entire simulation study, data were generated from model [1] with "known" population values for the fixed effects taken as $(\delta_1,\ \delta_2,\ \delta_3)'$=(5.051236, 13.86703, 0.8486555)'. The random factor vectors, $(\eta_{1i},\ \eta_{2i,}\ \eta_{3i})'$, were generated according to a centred multivariate normal and the residuals e_{ij} were also generated according to a normal model with zero mean.

In the first part of the simulation study, the covariance matrix or the generated random factors was, *always*:

$$D = \begin{pmatrix} 51.88633 & -1.0498620 & -0.05460614 \\ & 15.8465000 & -0.04587260 \\ & & 0.01362791 \end{pmatrix}$$

and residuals were *always* independent with variance *always* characterized by the same dispersion parameter, $\sigma = 0.939921$.

Each one of generated data tables was processed to fit a nonlinear mixed model like [1] via the LB method, assuming *in the fitting process* one of the following structures of the covariance matrix of random effects:

i. Independent random effects: thus, their covariance matrix is diagonal, and is denoted as *D_ind*.

ii. Block-diagonal: the first two random effects, $(\eta_{1i},\eta_{2i,})'$, have equal variance and are independent of the third one, η_{3i}. This can be rephrased by saying that the covariance matrix of the random effects D can be partitioned into four blocks as follows:

$$D = \begin{bmatrix} D_{11} & D_{12} & 0 \\ D_{21} & D_{11} & 0 \\ 0 & 0 & D_{33} \end{bmatrix}.$$

The $(\eta_{1i},\eta_{2i,})'$ form a 2×2 block of random effects with compound symmetry covariance matrix, and the η_{3i} forms another 1×1 block with an unstructured covariance matrix.

The corresponding covariance matrix is denoted as D_bck in the results.

iii. Unstructured covariance matrix: a general covariance matrix for the random effects (i.e. random effects have an arbitrary covariance structure) is assumed. This case is denoted as D_unst.

Each one of the preceding assumptions on the covariance structure of the random effects was combined, in the fitting process, with one of the following assumptions for the residuals correlation structure, used to model the within-group correlation:

i. Independent residuals,

ii. Autoregressive, AR(1), residuals, and

iii. Autoregressive moving average of order 1, MA(1), residuals.

The fitting conditions defined by D_unst and independent residuals were introduced to have a reference condition.

In the second part of this simulation study, the simulated data sets were generated according to three possible "true" structures of the random effects covariance matrix (instead of the general matrix D of the first part of the simulation study) that were combined with each one of three possible correlation structures for the residuals.

The possible structures for the covariance matrix were:

i. Uncorrelated random effects:

$$D\_ind = \begin{pmatrix} 41.889 & 0.00000 & 0.000000000 \\ 0.000 & 17.25313 & 0.000000000 \\ 0.000 & 0.00000 & 0.004827908 \end{pmatrix}.$$

ii. Block-diagonal:

$$D\_bck = \begin{pmatrix} 20.25 & 0.00 & 0.0000 \\ 0.00 & 20.25 & 0.0000 \\ 0.00 & 0.00 & 0.0064 \end{pmatrix}.$$

iii. Unstructured covariance matrix:

$$D\_unst = \begin{pmatrix} 48.00 & -1.00 & -0.05 \\ -1.00 & 16.00 & -0.05 \\ -0.05 & -0.05 & 0.02 \end{pmatrix}.$$

The residuals were always generated according to a stationary gaussian process with zero mean, but with correlation specified by one of the following structures:

i. Uncorrelated: for each individual i, $cor(e_{ij}, e_{ij+1}) = 0$, $j = 1,\ldots,n_i - 1$.

ii. Auto-regressive of order 1, AR (1):

$$cor(e_{ij}, e_{ik}) = \phi^{|j-k|}, \quad j,k = 1,\ldots,n_i; \text{ with } \phi = 0.2740697.$$

iii. Moving average of order 1, MA(1):

$$cor(e_{ij}, e_{ij+1}) = \alpha_1 \Big/ (1 + \alpha_1^2), \text{ where } e_{ij} = \alpha_1 e_{ij-1} + u_{ij}; \text{ with } \alpha_1 = 0.2095072.$$

To sum up, there were 18 simulation experimental conditions. In the first part of the simulation study, data *always* generated according to the same parameters (defining an unstructured covariance matrix for the random factors and uncorrelated residuals) were analysed according to 3x3=9 possible structure assumptions. In the second part of the study, data were generated according to 9 possible dependency structures for random factors and residuals, but always analysed assuming an unstructured covariance matrix and no residuals correlation.

The simulated random effects were generated according to an expression equivalent to $\eta_i = LH_i$, where H_i stands for a standardised version of the vector of random effects, generated from a normal distribution with zero mean and unit variance, and L stands for the lower

triangular matrix resulting from the Cholesky decomposition of a covariance matrix D with one of the possible structures cited previously. The simulated residuals for each individual were generated by an expression equivalent to $e_i = \sigma \Gamma u_i$, where u_i stands for a standardized vector of independent and identically distributed residuals, generated from the normal distribution with zero mean and unit variance; and Γ is the correlation matrix with the possible structures also cited and discussed above.

In order to obtain each new simulated response in accordance with model [1], each generated vector of random factors and residuals was added to the expected response. The generation process is described with more detail in El Halimi et al.(2004). For each one of the 18 experimental conditions, the entire process was repeated $N = 1000$ times.

For each generated data table, a nonlinear mixed model was fitted using the *nlme* S-Plus library, providing a set of (simulated) estimated values $\hat{\delta}$, $\hat{\eta}$, $\hat{D}$ and $\hat{\sigma}$ obtained according to the LB method. The bias and the mean square error (MSE) of these estimators was estimated from each series of 1000 simulated values, together with the true coverage of the associated asymptotic confidence intervals, always computed at a nominal 95% level.

For some simulated data tables the LB estimation methods did not converge. In these cases, we discarded the problematic pseudodata set and generated a completely new table, until convergence was reached. The possible consequences of this strategy are discussed in El Halimi et al.(2004).

3 Simulation results

a. Results for fixed effects

Table 1 and Figure 1 display the simulation results for fixed effects in the first series of simulations, where the means over 1000 simulations of δ_1 are close to the true value for all simulations runs. The bias is negative and significantly different from 0, but considerably small

(especially when the residuals are independent). This suggests that, on average, they will slightly underestimate the true parameter value, and shows a similar tendency under each one of the covariance structure of random effects. The same patterns are also seen for the coverage probability measures and the average widths of the intervals. All simulations produce nearly identical results in terms of MSEs. With respect to its true coverage probability, the results are quite similar when the model is fitted under the *D_ unst* and *D_ ind* model assumptions; both achieving the same maximum coverage, 93.8%, when the residuals are assumed independent, but the observed coverages are also quite similar under the other assumptions for the residuals. The coverages for *D_ bck* demonstrate the imprecise nature of the corresponding intervals, with a maximum coverage of 85.5% under the assumption of independent residuals and similar but lower coverages under the remaining assumptions on the residuals. In all conditions the intervals are appreciably equitailed, in the sense that the non coverage cases are nearly symmetrically distributed, approximately one half of the times the true parameter value is at left of the confidence interval, and the other half of the times at right.

For the δ_2 parameter the results shown in Table 1 suggest also a negative bias. Again, the bias and the MSE values are nearly identical, except for (*D_ bck*, AR (1)) conditions, where the MSE is roughly 50% larger than under other conditions. The coverage results are nearly inverted: the confidence intervals have its lowest coverage when the covariance matrix of random effects has the *D_ ind* and *D_ unst* structures, and the largest for the *D_ bck* structure, achieving maximum coverage, 98.2%, under (*D_ bck*, Independent) and (*D_ bck*, MA(1)) conditions. The confidence intervals are highly asymmetric, and the interval widths are similar in all conditions, except under *D_ bck*, characterized by large interval lengths.

With respect to bias, the estimates of δ_3 are good and not affected by wrong assumptions on the structures of random effects and residuals. On the other hand, the confidence intervals are very sensible (and always very incorrect) to these assumptions. The interval widths are similar, and the intervals exhibit considerable asymmetry. The maximum coverage, 79.3%, occurs at *D_ bck*

/ MA(1) conditions.

In general, all the confidence intervals for the fixed-effects parameters seem to be more affected by the structure of the covariance matrix random effects than by the correlation structure of the residuals.

The results of the second part of the simulation study are summarized in Table 2 and in Figure 2. In all cases, the point estimates of δ remain virtually unaffected by the choice of the residuals correlation structure and are slightly affected by the choice of the random effects covariance structure. Table 2 confirms that the averages of the fixed effects estimates are close to the true parameters for all simulations.

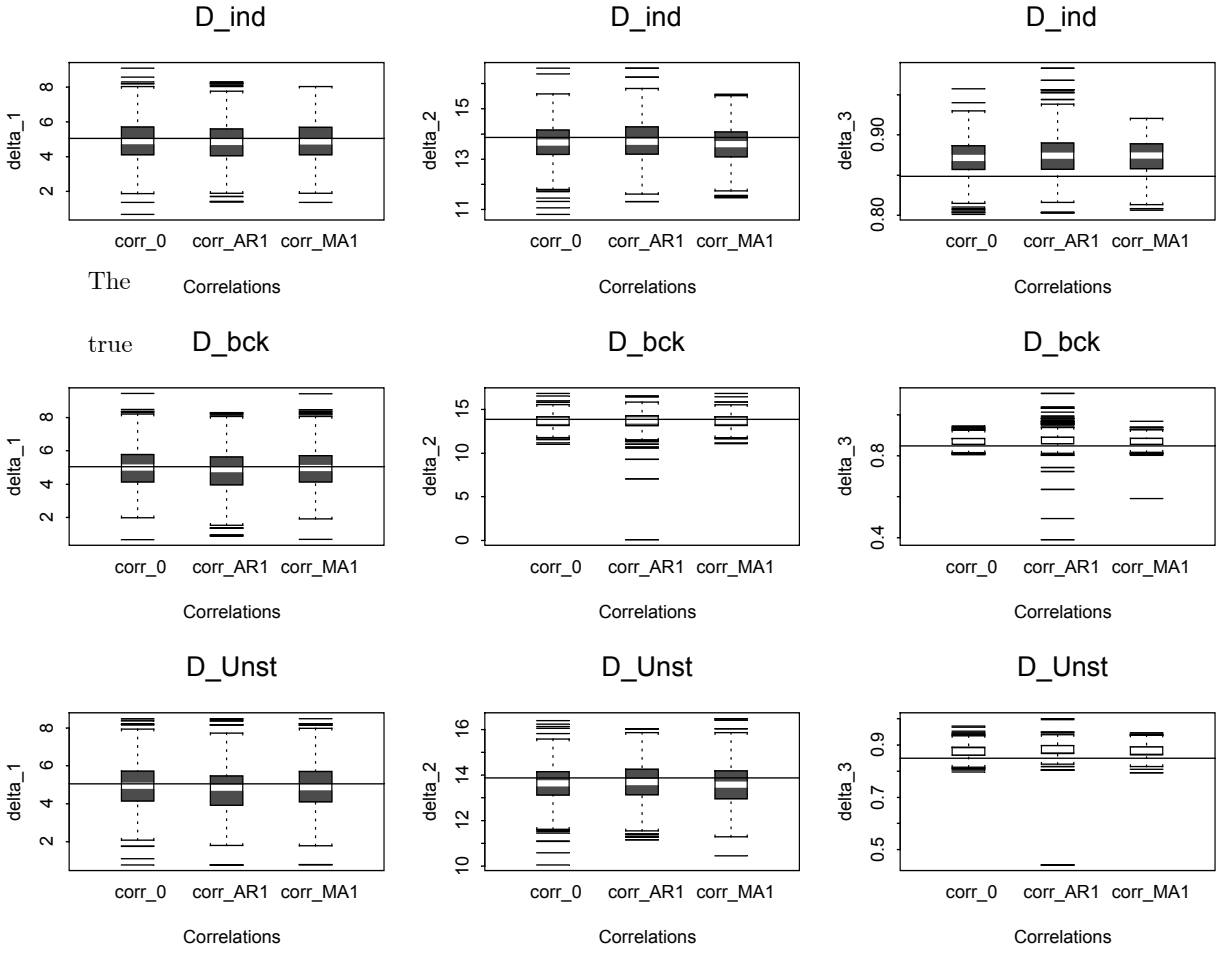

Figure 1. Breast cancer simulation scenario. Box-plots of 1000 simulation replicates of fixed effect estimates under different structures of D and correlation structures (First part of the simulation study)

coverage of the confidence intervals based on the LB-method is not always adequate, even under a correct specification of the covariance structure of random effects and residuals. It depends, mainly, on the specific model parameters. In any case, regarding each one of the assumed random effects covariance structures, we remark that the confidence interval coverage probabilities change with the structure of the matrix D and perform poorly when D_bck is used to generate data. On the other hand, it seems not to be considerably affected by the correlation structure of residuals.

Table 1: Simulation results for the breast-cancer model under different dependence structures for random effects and residuals (simulation study 1st part)

		δ_1						
Structure of D	Residuals	Mean	Bias±C.I$_{\text{Bias}}$	MSE	PROB. LOW	COV. PROB	PROB. UPP	Average width
D_ind	Independent	4.890293	-0.160943±0.07338365	1.426302	2.1%	93.8%	4.1%	4.470603
	AR(1)	4.836839	-0.2143969±0.07536222	1.522899	3.1%	92.1%	4.8%	4.407850
	MA(1)	4.891355	-0.1598807±0.07399343	1.446481	3.6%	92.3%	4.1%	4.490843
D_bck	Independent	4.981798	-0.06943841±0.0750465	1.469406	6.1%	85.5%	8.4%	3.605085
	AR(1)	4.820172	-0.2310643±0.08055745	1.740972	6.8%	81.2%	12%	3.515189
	MA(1)	4.973567	-0.07766946±0.07423818	1.439237	7.1%	85.2%	7.7%	3.607577
D_unst	Independent	4.959736	-0.09150034±0.07311737	1.398627	2.9%	93.8%	3.3%	4.469127
	AR(1)	4.743657	-0.3075795±0.07441145	1.534507	2.6%	92.1%	5.3%	4.376982
	MA(1)	4.882728	-0.168508±0.07377075	1.443608	2.3%	93.3%	4.4%	4.443382
		δ_2						
D_ind	Independent	13.6556	-0.2114322±0.04648119	0.6065372	2.9%	86.9%	10.2%	2.419676
	AR(1)	13.7077	-0.1593291±0.0499392	0.6739253	3.3%	87.7%	9%	2.387804
	MA(1)	13.5767	-0.2903252±0.04663594	0.6487374	3%	86.5%	10.5%	2.415387
D_bck	Independent	13.68257	-0.1844598±0.04613696	0.5875683	0.5%	98.2%	1.3%	3.661344
	AR(1)	13.61481	-0.2522202±0.06957238	1.322329	1.4%	92%	6.6%	3.599156
	MA(1)	13.67776	-0.1892706±0.04545654	0.5731596	0.3%	98.2%	1.5%	3.66568
D_unst	Independent	13.62828	-0.2387503±0.04928767	0.6887295	2.9%	86.6%	10.5%	2.461421
	AR(1)	13.64761	-0.2194167±0.05337408	0.7889661	4.2%	83.3%	12.5%	2.372432
	MA(1)	13.56978	-0.2972548±0.05398211	0.8461577	2.3%	84.6%	13.1%	2.474254
		δ_3						
D_ind	Independent	0.8713735	0.02271801±0.001403027	0.001028008	22.3%	77.3%	0.4%	0.08177437
	AR(1)	0.8749249	0.02626941±0.001611147	0.001365113	26.4%	73.6%	0%	0.08163308
	MA(1)	0.8736767	0.02502116±0.00139684	0.001132438	29.2%	70.8%	0%	0.08218339
D_bck	Independent	0.8697888	0.02113329±0.001365946	0.0009318155	20.8%	79%	0.2%	0.08095256
	AR(1)	0.8763528	0.02769726±0.002709488	0.002676234	29.5%	69.6%	0.9%	0.08205351
	MA(1)	0.8697713	0.02111575±0.001477302	0.001013409	20.6%	79.3%	0.1%	0.08339257
D_unst	Independent	0.8751281	0.02647256±0.001485831	0.001274902	27%	72.9%	0.1%	0.08439246
	AR(1)	0.8813267	0.03267124±0.002452305	0.002631287	36.8%	62.7%	0.5%	0.08965067
	MA(1)	0.8784579	0.02980238±0.001465699	0.001446835	31.3%	68.5%	0.2%	0.08541293

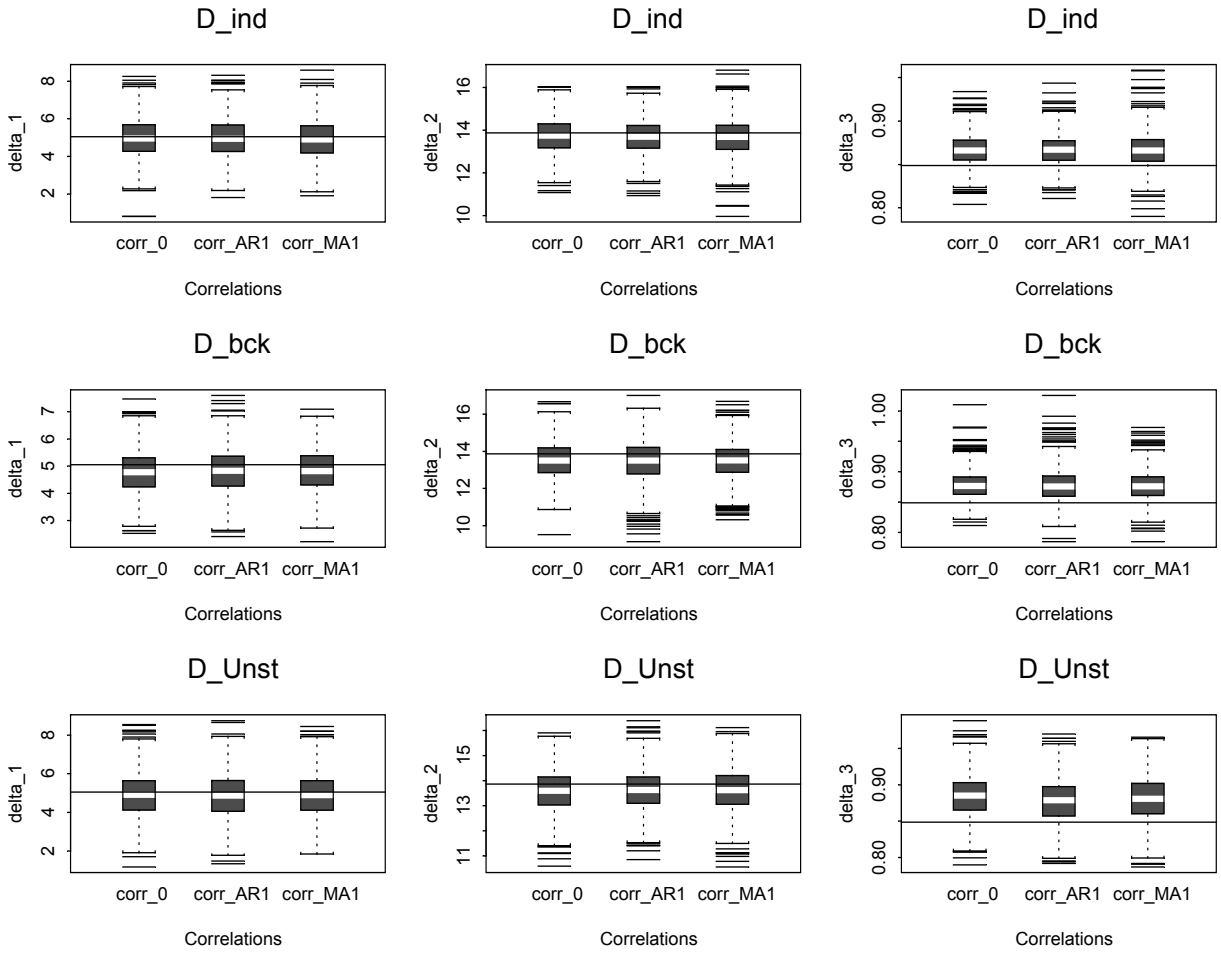

Figure 2. Breast cancer simulation scenario. Box-plots of 1000 simulation replicates of fixed effect estimates under different structures of D and correlation structures (Second part of the simulation study)

Table 2: Simulation results for the breast-cancer model under different dependence structures for random effects and residuals (simulation study 2nd part)

		δ_1						
Structure of D	Residuals	Mean	Bias±C.I$_{\text{Bias}}$	MSE	PROB. LOW	COV. PROB	PROB. UPP	Average width
D_ind	Independent	4.972995	-0.0782413±0.06598653	1.138428	2.9%	93.6%	3.5%	4.055087
	AR(1)	4.950308	-0.100928±0.06366188	1.064118	2.2%	94%	3.8%	4.022737
	MA(1)	4.902534	-0.1487024±0.06559291	1.14095	2.2%	93%	4.8%	4.03939
D_bck	Independent	4.78057	-0.2706657±0.04801458	0.672774	1.7%	91.1%	7.2%	2.871522
	AR(1)	4.809881	-0.2413553±0.04955735	0.696912	1.4%	91.3%	7.3%	2.919448
	MA(1)	4.821342	-0.2298941±0.0468499	0.623634	0.6%	92.7%	6.7%	2.896072
D_unst	Independent	4.887868	-0.1633685±0.07022588	1.309161	2%	93.2%	4.8%	4.305583
	AR(1)	4.852252	-0.1989843±0.0699953	1.313658	1.9%	93.7%	44%	4.342266
	MA(1)	4.904378	-0.1468583±0.06974602	1.286572	2.7%	93%	43%	4.352609
		δ_2						
D_ind	Independent	13.86703	-0.1537563±0.04941836	0.6587233	3.9%	87.4%	8.7%	2.531632
	AR(1)	13.69579	-0.1712411±0.04902582	0.6543568	3.8%	87.7%	8.5%	2.524353
	MA(1)	13.67516	-0.1918734±0.05365122	0.7853511	5.2%	84.9%	9.9%	2.533162
D_bck	Independent	13.5189	-0.3481297±0.06002156	1.05804	4.5%	79.6%	15.9%	2.691222
	AR(1)	13.48332	-0.3837145±0.06705982	1.316677	5.2%	76.9%	17.9%	2.679371
	MA(1)	13.48008	-0.3869503±0.06071217	1.108259	3.6%	81%	15.4%	2.689977
D_unst	Independent	13.57039	-0.2966377±0.05084926	0.760386	3.3%	82.8%	13.9%	2.447999
	AR(1)	13.60609	-0.260937±0.05064817	0.7351727	3.6%	84.2%	12.2%	2.458311
	MA(1)	13.61227	-0.2547586±0.05246764	0.7807756	3.3%	83.9%	12.8%	2.448161
		δ_3						
D_ind	Independent	0.8665674	0.017912±0.001100081	0.000636	29.3%	70.1%	0.6%	0.05623834
	AR(1)	0.8666122	0.017959±0.001068346	0.000619	26.5%	72.9%	0.6%	0.05705633
	MA(1)	0.8662308	0.017575±0.001220561	0.000696	25.4%	74.1%	0.5%	0.06123209
D_bck	Independent	0.8779196	0.029264±0.001440842	0.001396	40.1%	59.8%	0.1%	0.06746345
	AR(1)	0.8766831	0.028028±0.001481441	0.001481	37%	62.4%	0.6%	0.07307422
	MA(1)	0.8486555	0.028395±0.001471719	0.001369	38.3%	61.2%	0.5%	0.07119195
D_unst	Independent	0.8842841	0.035628±0.03562864	0.002110	32.4%	67.5%	0.1%	0.09821503
	AR(1)	0.8779992	0.029343±0.001854973	0.001755	24.6%	75.3%	0.1%	0.1018569
	MA(1)	0.8486555	0.031735±0.001841488	0.001887	26.8%	72.9%	0.3%	0.09997221

b. Results for variance components

Figure 3 summarizes the main results of the first series of simulations, those testing the impact on the LB method on the random components when different, inadequate, structures of the covariance matrix of random effects and/or correlation structures of the residuals are imposed. Figure 3 displays the results for the ML estimations of the terms of the random factors covariance matrix, D, and for the standard deviation of the residuals, σ. The bias and the overall distribution of the estimates of the components of D, and the coverage of the corresponding asymptotic confidence intervals, depends on the parameter in question and on the imposed covariance of random effects, more than on the correlation structure of the residuals. In general terms, the inference on the components of D is even less robust than the inference on the fixed effects, but adequate under the (correct) assumption of unstructured covariance matrix. For all components of D, the coverage in the D_unst case always lies near the nominal 95% level, while the coverages under D_bck are very erratic and affected by both, the covariance of the random effects and the correlation of residuals. Clearly, this assumption on the structure of the covariance matrix is too strong when it is incorrect.

As expected, the results for the estimator of the standard deviation of the residuals, σ, show a clear dependency on the correlation structure of the residuals, rather than that of the random effects. The coverage of the asymptotic confidence intervals for this parameter are always very inadequate and range from 49.3% in the (D_bck, MA(1)) case to 42.9% in the (D_unst, AR(1)) case, with 45.3% of coverage under (D_unst, independent) conditions.

The results corresponding to the estimation of the random part in the second series of simulations are displayed in Figures 4 to 7, which are box-plots of the estimates of the variance components (D and σ) for all simulation runs. They can be interpreted in the same way as Figure 3. As expected, the median values of all the components of D change considerably with the true values of D used in the simulations, and the variability of the estimations of the D components changes considerably with the different assumed structures of D, especially for D_{23}

and D_{33}, but both the point estimates and the confidence intervals are correct when the process of estimation is performed assuming an unstructured covariance matrix and not its exact structure. The precision of the estimates of the covariance matrix is nearly not affected by incorrectly assuming independent residuals instead of the adequate correlation model for the residuals.

Finally, the variability of the point estimates of the parameter σ is affected by the true values and the dependence structure of both, the random effects and the residuals, but not its bias nor the true coverage of the asymptotic confidence intervals, that are quite correct in all conditions. That is, again the results are acceptable when the estimation process is performed under the assumption of unstructured covariance matrix and independent residuals, instead of assuming its correct structure.

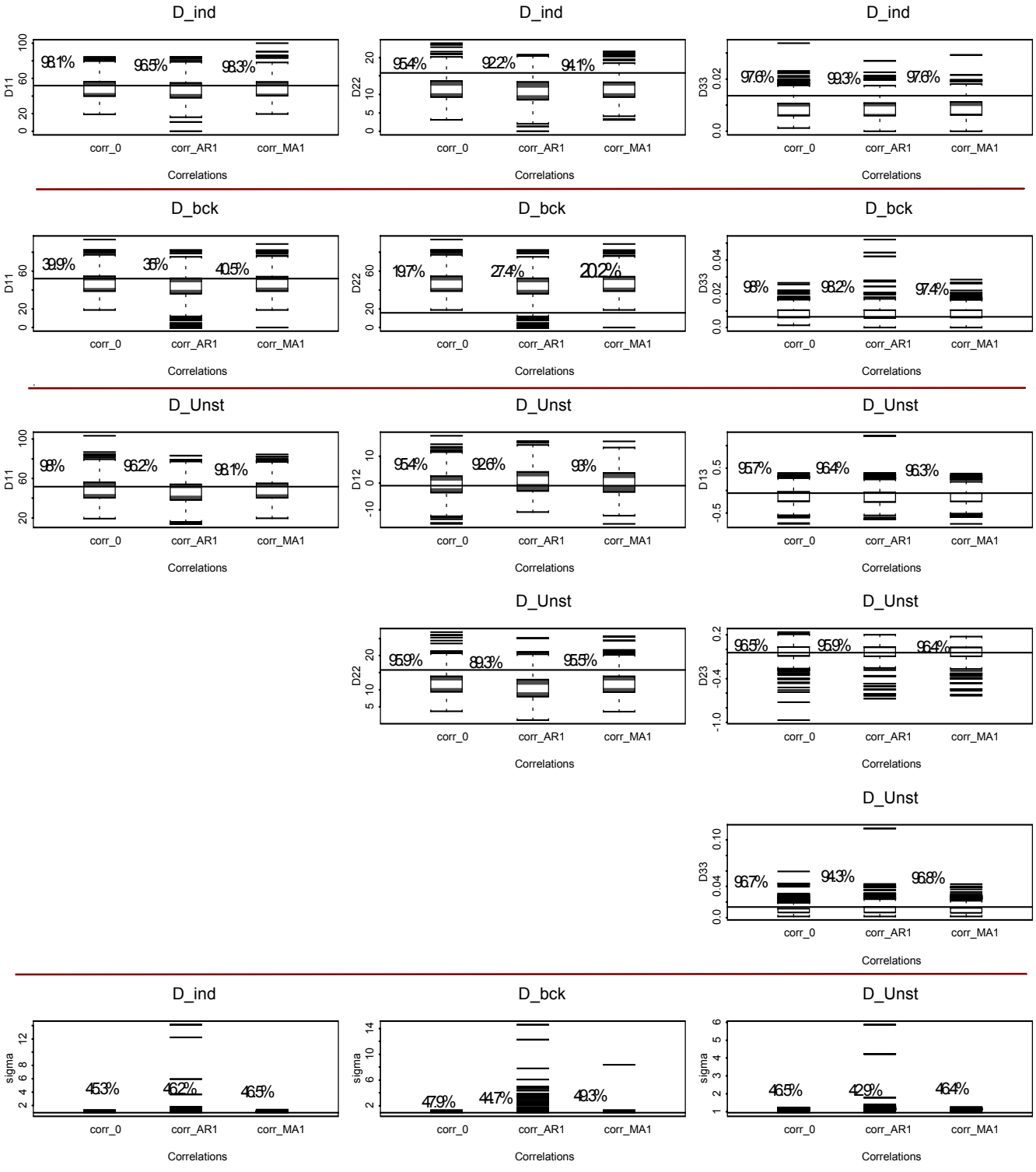

Figure 3. Breast cancer simulation scenario. Box-plots of 1000 simulation replicates of random components estimates under different structures of D and correlation structures (first part of the simulation study)

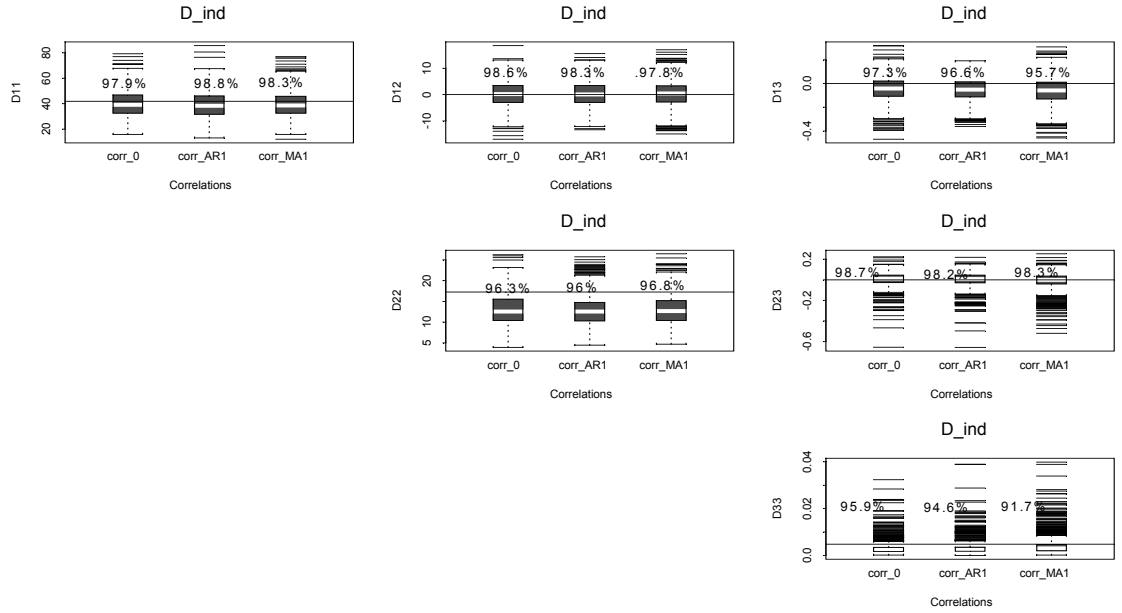

Figure 4. Breast cancer simulation scenario. Box-plots of 1000 simulation replicates of random effects covariance estimates under *D_ ind* and different correlation structures (simulation study 2nd part)

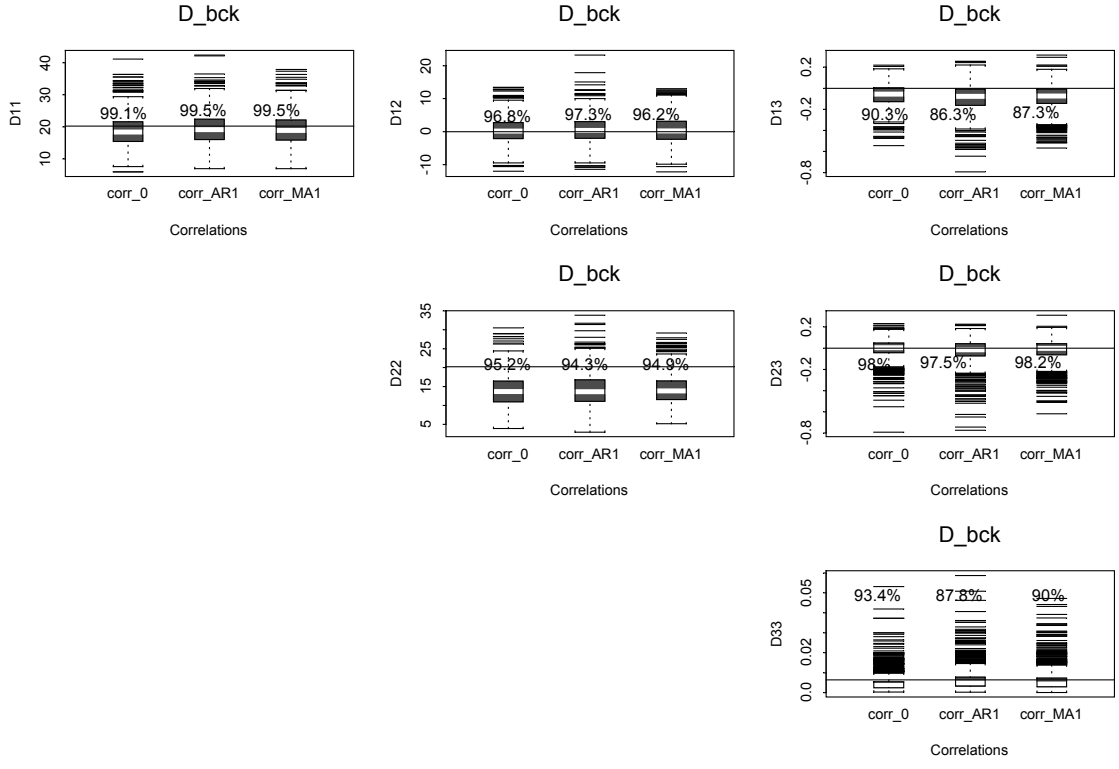

Figure 5. Breast cancer simulation scenario. Box-plots of 1000 simulation results of random effects covariance estimates under *D_ bck* and different structures of correlation (simulation study 2nd part)

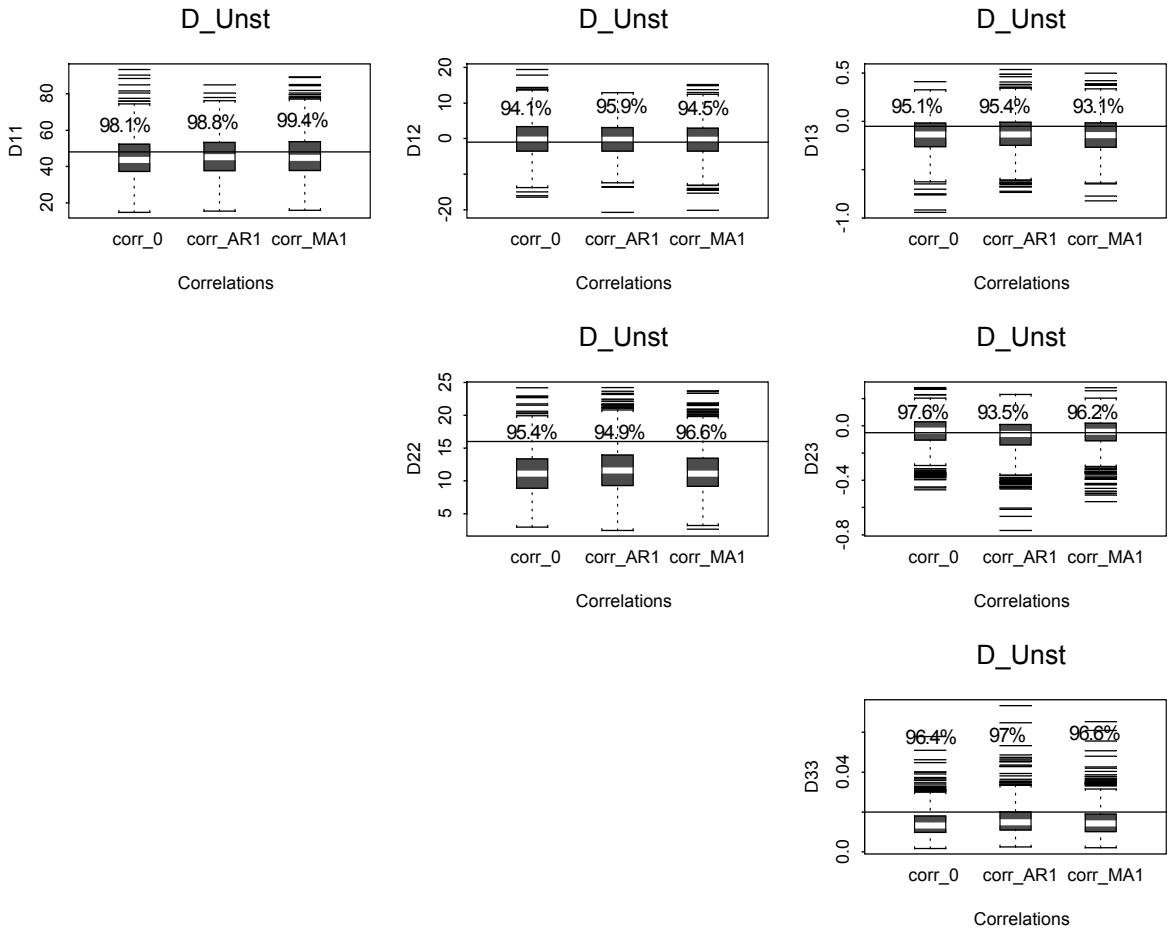

Figure 6. Breast cancer simulation scenario. Box-plots of 1000 simulation replicates of random effects covariance estimates under D *unst* and different structures of correlation (simulation study 2nd part)

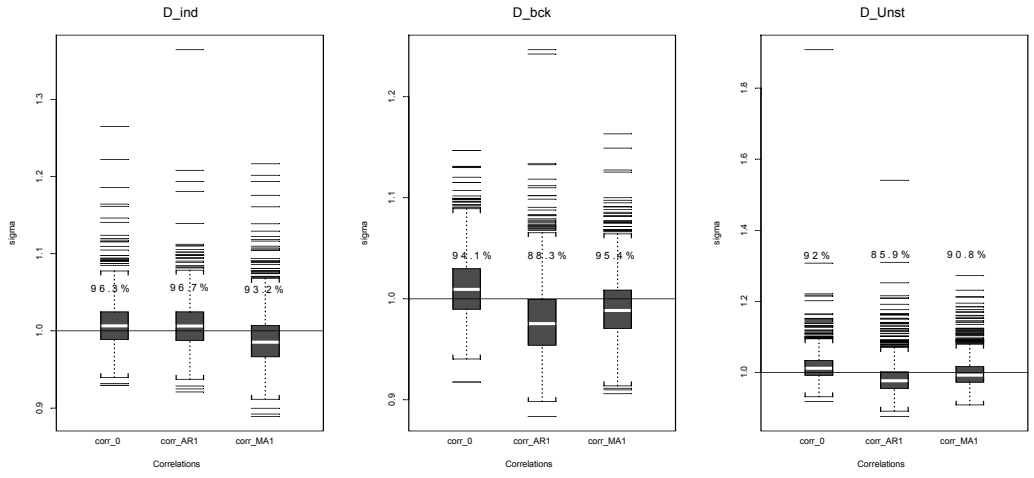

Figure 7. Breast cancer simulation scenario. Box-plots of 1000 simulation σ estimates under different variance-covariance structures of random effects and different residual correlation structures (simulation study 2nd part)

4 Soybean genotypes simulation

a. Simulation design

To give some insight on the "representativeness" of the above simulations and results, we performed a complementary simulation study based on the Soybean genotypes data and model, reported in Davidian and Giltinan (1995, p.7) and reanalysed by Pinheiro and Bates (2000). Using the same notation than in model [1], the base model to be simulated was:

$$y_{ij} = \frac{\left[\delta_1 + \eta_{1i}\right]}{1 + \exp\left[\left(\left[\delta_2 + \eta_{2i}\right] - t_{ij}\right) / \left[\delta_3 + \eta_{3i}\right]\right]} + e_{ij} \qquad [2]$$

where y_{ij} represents the average leaf weight in plant i, $i = 1, \ldots, 48$, at time t_{ij}. The random effects $\eta_i = (\eta_{1i},\ \eta_{2i},\ \eta_3)'$ are $(0,\ D)$ and the e_{ij} are $(0,\ \sigma^2)$ and are independent of the η_i. The following "population" parameters were taken,

$$\delta = (\delta_1,\ \delta_2,\ \delta_3)' = (19.26,\ 55,\ 8.4)$$

$$D = \begin{pmatrix} 25 & 2.50 & 4.00 \\ & 8.00 & 2.32 \\ & & 2.00 \end{pmatrix}; \qquad \sigma = 1.$$

The simulation study was also divided in the same two parts; the first one devoted to the consequences of fitting inappropriate restricted models (the same than in section 2) and the second one to the consequences of fitting under unrestricted conditions when data were generated according to some true structuring patterns. More concretely, the simulated datasets were generated according to each one of the nine possible combinations of the following covariance matrices for the random effects and correlation structures for the residuals:

i. Independent random effects:

$$D\_ind = \begin{pmatrix} 25 & 0 & 0 \\ 0 & 7.749999 & 0 \\ 0 & 0 & 0.8843354 \end{pmatrix}$$

ii. Block-diagonal: independent subgroups of random effects:

$$D\_bck = \begin{pmatrix} 9 & 0 & 0 \\ 0 & 9 & 0 \\ 0 & 0 & 9 \end{pmatrix}$$

iii. Unstructured covariance matrix:

$$D\_Unst = \begin{pmatrix} 31.5574859 & 0.2019679 & 0.1767219 \\ 0.2019679 & 20.1967910 & 0.2272130 \\ 0.1767219 & 0.2272130 & 5.0491977 \end{pmatrix}$$

and

i. Independent residuals: for each individual i, $cor(e_{ij}, e_{ij+1}) = 0, j = 1,\ldots,n_i$

ii. Auto-regressive process of order1, AR(1):

$$cor(e_{ij}, e_{ik}) = \phi^{|j-k|}, j,k = 1,\ldots,n_i; \text{ where } \phi = \text{-0.6195506}$$

iii. Moving average of order 1, MA (1):

$$cor(e_{ij}, e_{ij+1}) = \alpha_1 \Big/ (1+\alpha_1^2), \text{ where } e_{ij} = \alpha_1 e_{ij-1} + u_{ij}; \text{ and } \alpha_1 = \text{-0.9942504.}$$

For each one of the 18 simulation experimental conditions, 1000 data sets where generated according to the nonlinear mixed model [2]

b. Simulation results

Table 3 and Figure 8 summarise the results on the fixed parameters for the first part of this simulation study. The results depend on each specific parameter.

For the first parameter, δ_1, the differences between all simulation conditions with respect to mean, bias and mean square error (MSE) of the point estimates are small. The biases are negative and significantly different from zero, though small. The MSE values are also similar and small.

The main factor affecting the observed coverage of the confidence intervals is the (erroneously) assumed structure of the random effects covariance matrix. As may be expected, the best values are obtained for *D_unst*, with a maximum of 93.4% for *D_unst* combined with independent residuals. The worst observed coverages correspond to *D_bck*, with the lowest coverage, a 82%, corresponding to *D_bck* and MA(1) residuals. The confidence intervals are always markedly asymmetrical, with an inflated PROB.UPP value (that is, there is a clear tendency for the intervals to be located entirely at right of the true parameter value). The mean width of the intervals is in accordance with the coverage, with the shortest intervals corresponding to the *D_bck* case.

For the δ_2 and δ_3 parameters, the results on the mean value, bias and MSE of the point estimates are very similar to those reported for δ_1, with small, though significant, negative bias in all conditions. On the other hand, and not in complete agreement with intuition, the results on confidence intervals are nearly completely reversed. The best coverages are obtained under the *D_bck* assumption, with a maximum for *D_bck* and AR(1) residuals (97.2% for δ_2 and 95% for δ_3). The lowest coverages correspond to *D_unst*, with the minimum coverage associated to *D_unst* and independent residuals. The intervals are still markedly asymmetrical, with PROB.UPP inflated values.

Still for the fixed parameters, the results of the second part of the simulation study are summarized in Table 4 and Figure 9. As a general rule, the point estimates of the fixed effects remain virtually unaffected by the fact of performing the analysis under the assumption of independent residuals when, in fact, there is some kind of residuals correlation structure, and are only slightly affected by the assumption of unstructured random effects covariance, when in fact random effects have a given structure. Table 4 confirms that the averages of the fixed effects estimates are close to the true parameters for all simulations. The true coverage of the confidence intervals based on the LB-method is not always adequate, even under a correct specification of the covariance structure of random effects

and residuals. It depends on the specific model parameters, much more than in the true covariance structure of the random effects and even less in the correlation structure of the residuals.

Finally, Figure 10 summarises the results on the random effects for the first part of the simulation study, while Figures 11 to 14 summarise the results for the second part of the study. They consist in box-plots of the generated simulation estimates of the components of D and of σ, jointly with the estimated coverages of the asymptotic confidence intervals, at a 95% nominal level. As in the preceding simulation study, the worst results correspond to the case of inappropriately modelling under inadequate restricted structures (Figure 10) while the opposite situation has a much lesser impact.

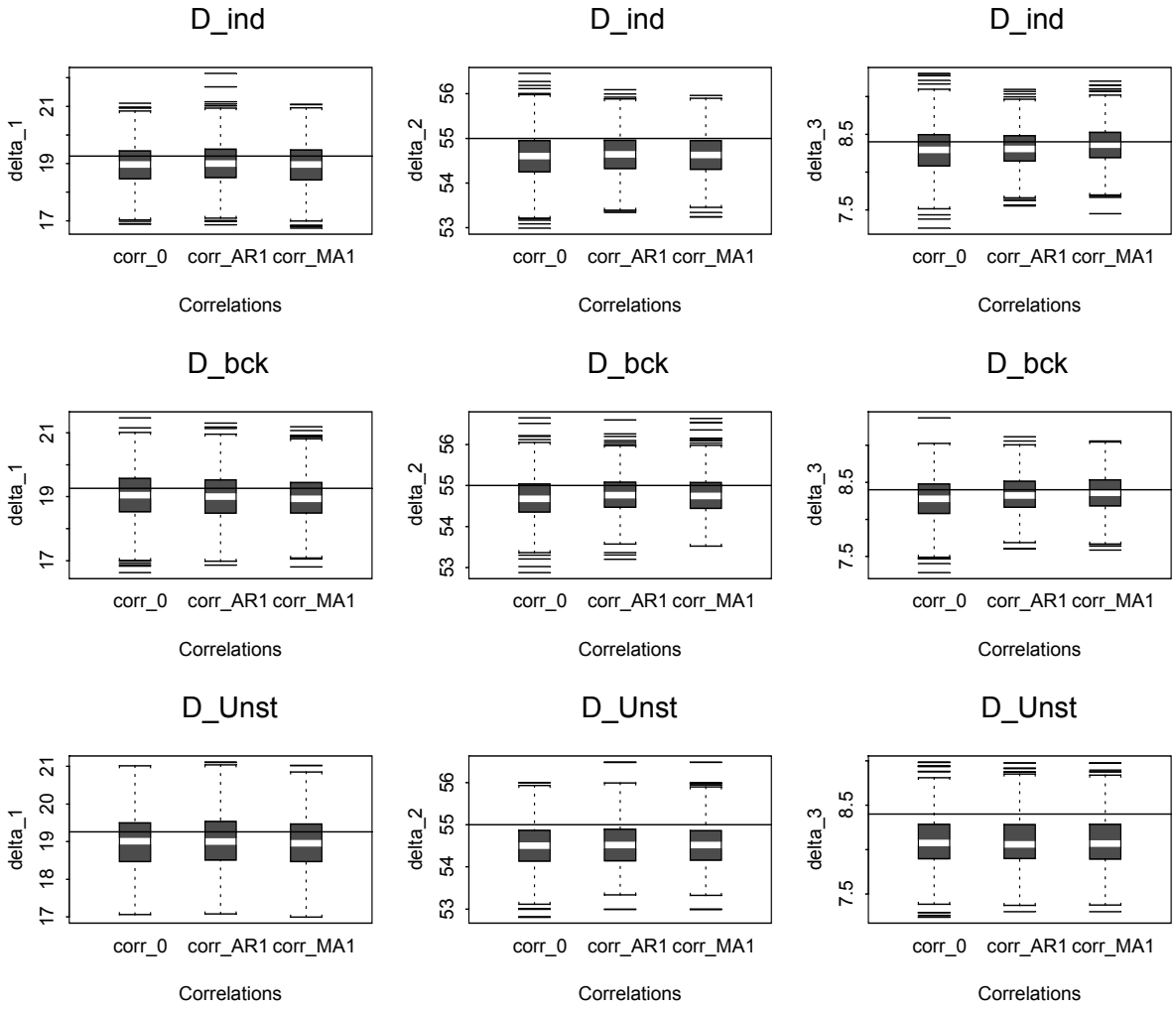

Figure 8. Box-plots for the results of 1000 simulations. Fixed effects estimates assuming different structures of the covariance matrix, D, and residuals correlation, for Soybean genotypes simulation scenario

Table 3: Simulation results for the Soybean genotypes model assuming different dependence structures for random effects and residuals

		δ_1						
Structure of D	Residuals	Mean	Bias±C.IBias	MSE	PROB. LOW	COV. PROB	PROB. UPP	Average width
D_ind	Independent	18.96579	-0.2942132±0.04566504	0.628838	1.4%	90.3%	8.3%	2.71004
	AR(1)	18.98863	-0.2713718±0.0459879	0.630425	1.8%	89.5%	7.3%	2.677696
	MA(1)	18.93715	-0.3228456±0.04659189	0.6687423	1.1%	89.3%	9.6%	2.69196
D_bck	Independent	19.04051	-0.2194925±0.04767421	0.6392217	2.6%	84.3%	13.1%	2.27268
	AR(1)	19.00413	-0.2558707±0.04589219	0.6131549	3.9%	82.3%	13.8%	2.201651
	MA(1)	18.96176	-0.2982434±0.04525658	0.6199689	3.4%	82%	14.5%	2.174162
D_unst	Independent	18.9922	-0.2678027±0.04541789	0.6081411	0.9%	93.4%	5.7%	2.914135
	AR(1)	19.01046	-0.249541±0.04591368	0.6104688	1.8%	92.1%	61%	2.915253
	MA(1)	18.9591	-0.3009036±0.04629004	0.6477652	1.1%	91.5%	7.4%	2.919399
		δ_2						
D_ind	Independent	54.6048	-0.3951971±0.03267328	0.4337932	0.7%	82.4%	16.9%	1.810568
	AR(1)	54.59309	-0.4069145±0.02901113	0.4402066	0.5%	82.2%	18.9%	1.580207
	MA(1)	54.63169	-0.3683053±0.03034018	0.3750298	0.6%	81.3%	18.1%	1.631354
D_bck	Independent	54.69015	-0.3098519±0.03242608	0.3694356	0.2%	95.7%	4.1%	2.498229
	AR(1)	54.77096	-0.2290404±0.02927588	0.2753406	0.3%	97.2%	2.5%	2.332616
	MA(1)	54.7555	-0.244497±0.03025544	0.2971096	0.5%	96.1%	3.4%	2.386578
D_unst	Independent	54.49707	-0.5029284±0.03486202	0.5689889	0.3%	79.5%	20.2%	1.951757
	AR(1)	54.5315	-0.4684997±0.03326956	0.5073296	0.7%	81.5%	17.8%	1.970419
	MA(1)	54.52909	-0.4709094±0.03280285	0.5015743	0.6%	82.3%	17.1%	1.969598
		δ_3						
D_ind	Independent	8.29996	-0.100040±0.01870675	0.10101	3.3%	87.2%	9.5%	0.9827692
	AR(1)	8.307381	-0.092619±0.01537698	0.106427	2.8%	85.3%	9.8%	0.8019704
	MA(1)	8.360465	-0.03953548±0.01578645	0.06637007	3.7%	89%	7.3%	0.8353948
D_bck	Independent	8.277938	-0.1220619±0.01854038	0.1042895	1.8%	87.3%	10.9%	0.9880616
	AR(1)	8.336482	-0.0635182±0.01532168	0.0650818	2.8%	89.5%	7.7%	0.816669
	MA(1)	8.350087	-0.04991292±0.01623579	0.07083428	3.9%	86.3%	9.8%	0.8251872
D_unst	Independent	8.089291	-0.310709±0.01931481	0.193554	0.6%	77.6%	21.8%	1.131231
	AR(1)	8.090309	-0.3096906±0.01855465	0.1854363	0%	80.7%	19.3%	1.129665
	MA(1)	8.09188	-0.3081204±0.01824214	0.1814758	0%	81.5%	18.5%	1.13002

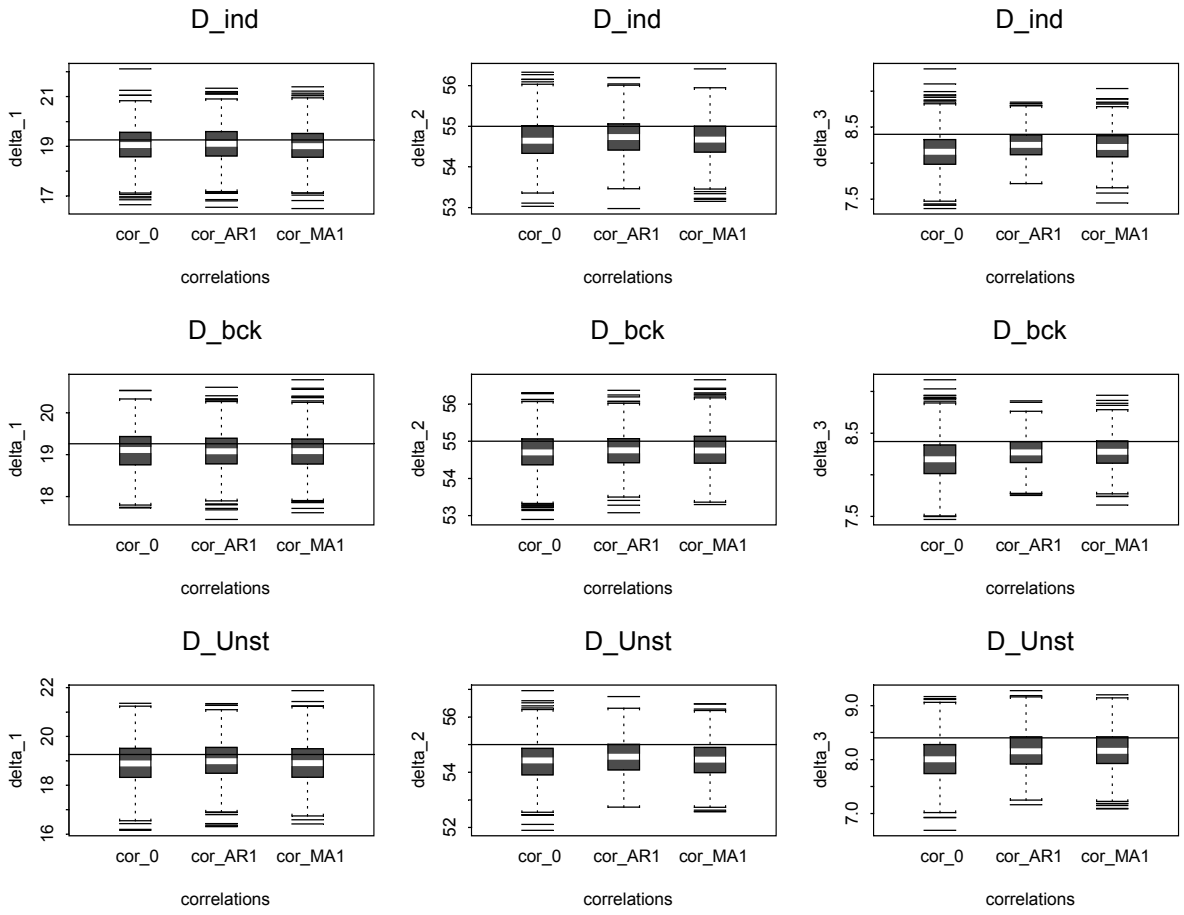

Figure 9. Box-plots for the results of 1000 simulations. Unrestricted fixed effects estimates for data generated under different structures of the covariance matrix, D, and residuals correlation. Soybean genotypes scenario

Table 4: Simulation results for Soybean genotypes model. Data generated according to different of covariance matrix and correlation structures

		δ_1						
Structure of D	Residuals	Mean	Bias±C.IBias	MSE	PROB. LOW	COV. PROB	PROB. UPP	Average width
D_ind	Independent	19.05448	-0.2055249±0.04558582	0.582638	1.1%	94%	4.9%	2.909964
	AR(1)	19.09658	-0.1634161±0.04548594	0.564198	1.7%	94%	4.3%	2.843851
	MA(1)	19.0362	-0.2238025±0.04533821	0.557341	1.2%	94.1%	4.7%	2.855609
D_bck	Independent	19.09566	-0.1643445±0.0300166	0.261311	1%	92.8%	6.2%	1.868798
	AR(1)	19.08624	-0.1737575±0.02823867	0.237560	1.3%	92.7%	6%	1.752299
	MA(1)	19.08849	-0.1715104±0.02834895	0.238407	1.9%	92.4%	5.7%	1.782298
D_unst	Independent	18.91455	-0.3454477±0.05340997	0.861153	1.2%	91.4%	7.4%	3.247077
	AR(1)	19.00475	-0.2552507±0.05040407	0.725822	1.3%	93.6%	5.1%	3.202265
	MA(1)	18.92533	-0.3346746±0.05407979	0.7842382	0.9%	92.2%	6.9%	3.183286
		δ_2						
D_ind	Independent	54.67918	-0.3208238±0.03182381	0.3662928	1%	88.9%	10.1%	1.9651
	AR(1)	54.73897	-0.2610341±0.02950031	0.2942236	0.5%	88.4%	11.1%	1.74265
	MA(1)	54.68726	-0.3127378±0.03052397	0.3277257	0.2%	89%	10.7%	1.8092
D_bck	Independent	54.69736	-0.302641±0.03325201	0.3791255	0.9%	89%	10.1%	2.040034
	AR(1)	54.75664	-0.2433551±0.02997396	0.2928587	1%	90.7%	8.3%	1.830022
	MA(1)	54.7616	-0.2384041±0.03278227	0.3363042	1.6%	88.6%	9.8%	1.908499
D_unst	Independent	54.396	-0.6039978±0.0450599	0.8928133	0.4%	84.1%	15.5%	2.78491
	AR(1)	54.57017	-0.4298255±0.04226079	0.6491889	0.4%	88%	11.6%	2.619695
	MA(1)	54.45269	-0.5473101±0.04403028	0.7451547	0.5%	86.8%	12.8%	2.654754
		δ_3						
D_ind	Independent	8.161801	-0.23820±0.01635497	0.126298	0.6%	80.9%	18.5%	0.9480518
	AR(1)	8.256304	-0.143697±0.01252	0.06137	0.9%	85%	14.1%	0.7293738
	MA(1)	8.232626	-0.167374±0.01353964	0.073253	0.7%	83.8%	15.5%	0.7712214
D_bck	Independent	8.190489	-0.209511±0.01543366	0.105838	0.7%	83.7%	15.6%	0.9447332
	AR(1)	8.270736	-0.129264±0.01142019	0.050625	0.2%	87.2%	12.6%	0.706781
	MA(1)	8.274229	-0.125771±0.012046	0.053553	0.5%	89%	10.5%	0.7573112
D_unst	Independent	8.007475	-0.392525±0.02390434	0.302671	0.1%	79.5%	20.4%	1.46326
	AR(1)	8.170378	-0.229622±0.0220047	0.178643	0.3%	86.9%	12.8%	1.343985
	MA(1)	8.166625	-0.2333746±0.02343736	0.1807237	0.5%	89%	10.5%	1.357662

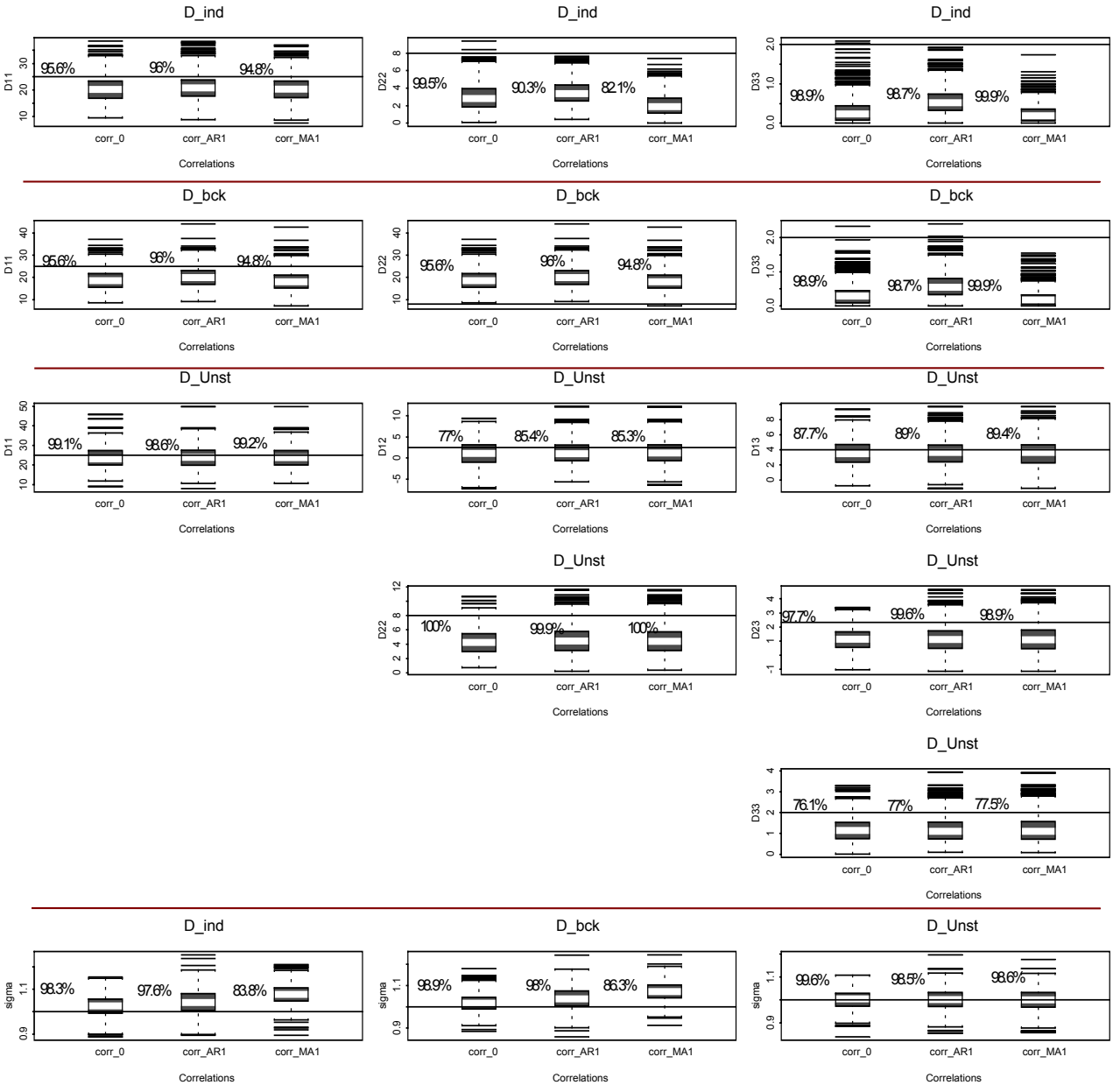

Figure 10. Random components estimates assuming different structures of the covariance matrix, D, and residuals correlation, when data are generated according to an unstructured covariance and uncorrelated residuals. Soybean genotypes scenario

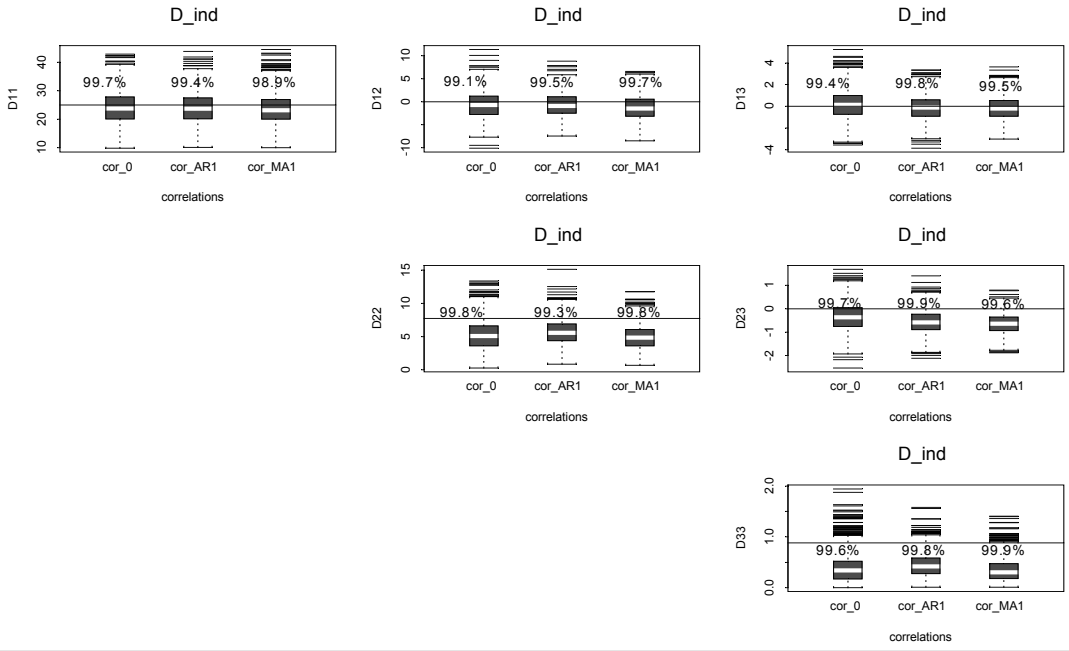

Figure 11. Random effects covariance estimates assuming unstructured covariance and uncorrelated residuals for data generated with independent random effects and different error correlation structures for Soybean genotypes scenario

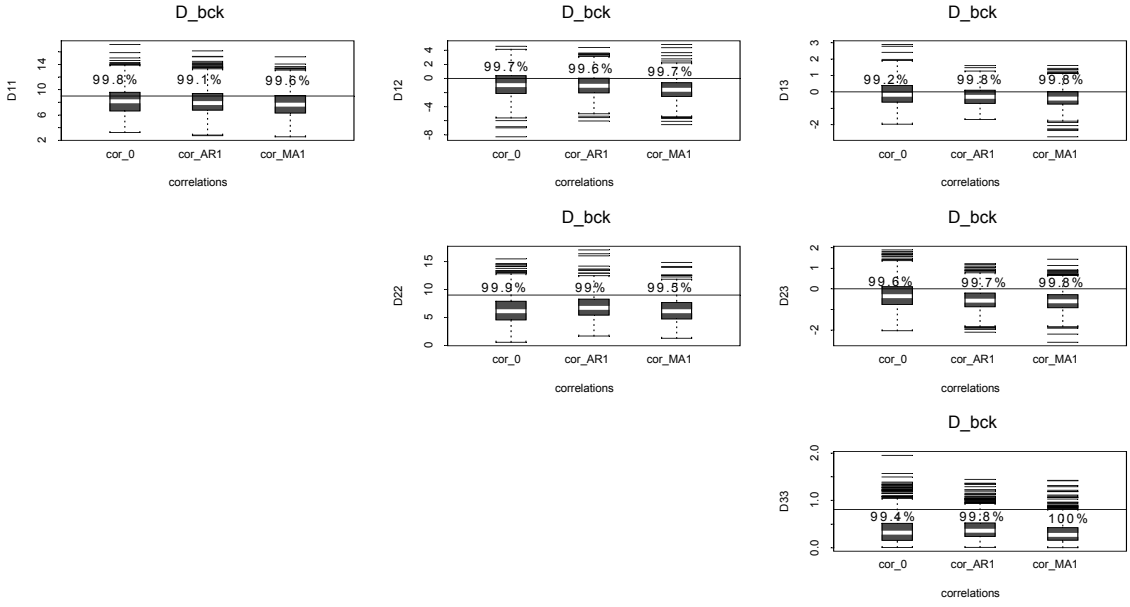

Figure 12. Random effects covariance estimates assuming unstructured covariance and uncorrelated residuals for data generated with blocked structure of D and different error correlation structures for Soybean genotypes

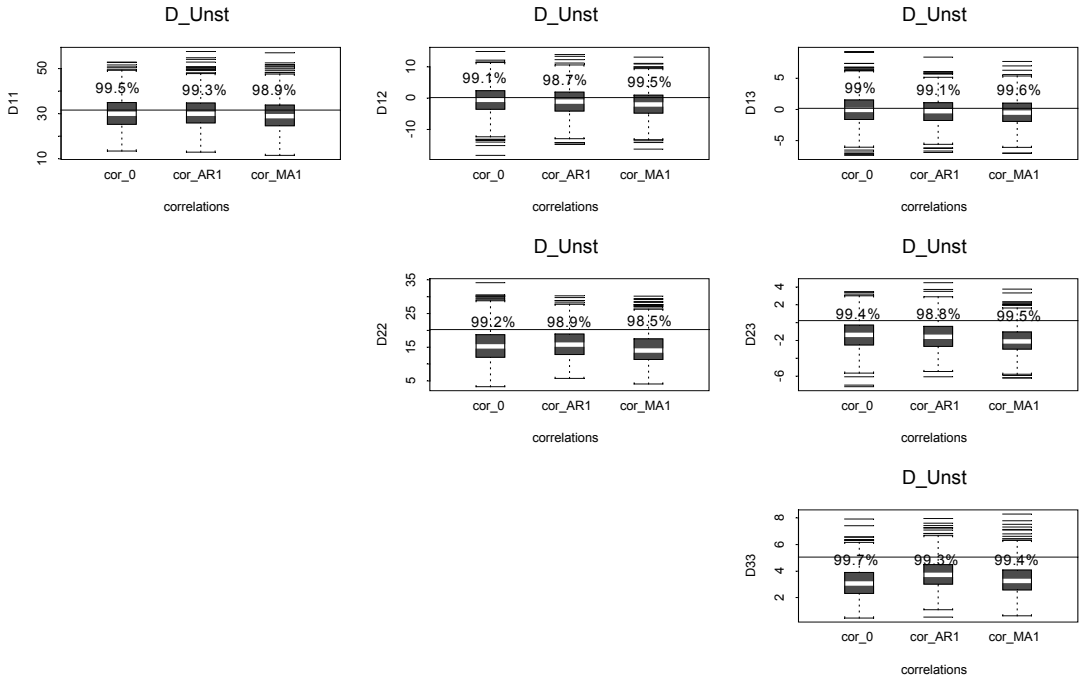

Figure 13. Random effects covariance estimates assuming unstructured covariance and uncorrelated residuals for data generated with unstructured D and different error correlation structures for Soybean genotypes scenario

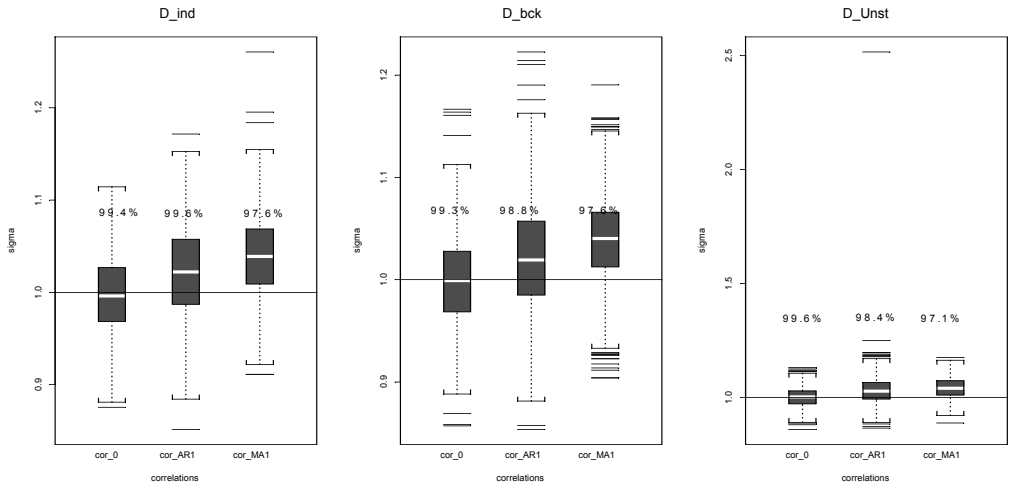

Figure 14. Box-plots of 1000 simulation σ estimates assuming unstructured covariance and uncorrelated residual for data generated with different structures D and different residuals correlation patterns for Soybean genotypes scenario

5 Discussion and conclusions

Though preliminary, the results of the two simulation studies reported in this paper suggest a general picture of the consequences of misspecifying the structure of the covariance matrix of the random effects and the residuals correlation, when fitting nonlinear mixed models.

The bias and MSE of the model parameters point estimates are not, in general, greatly affected by these misspecifications. The LB method performs well with respect to these measures and in a quite robust way. The more problematic results concern the true coverage of the asymptotic confidence intervals for the parameters, in agreement with similar studies devoted to the effects of invalid assumptions on the distributional form of random effects and/or residuals, like El Halimi et al. (2004). Even for moderately large sample sizes and large number of observations inside each experimental unit, frequently the true coverage does not correspond to the nominal level, and many times is smaller. Especially for fixed parameters, the observed coverage is not adequate even under valid assumptions. In any case, and ignoring other criteria like the convenience of avoiding over parameterised models, it is worst to erroneously assume some structure for the covariance matrix of the random effects than do not assume any structure when this would be adequate.

Finally, it is worth pointing that the results greatly depend on the specific parameters of the model (and on specific parameterisations of the same model), so it is difficult to take general conclusions.

Acknowledgements

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