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Modelling spatial and temporal correlation in multi-assessment perennial crop variety selection trials using a multivariate autoregressive model

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ABSTRACT

Context. Perennial crop variety selection trials are often conducted over several seasons or years. These field trials often exhibit spatial correlation between plots. When data from multiple assessment times are analysed, it is necessary to account for both spatial and temporal correlation. A current approach is to use linear mixed models with separable spatial and temporal residual covariance structures. A limitation of these separable models is that they assume the same spatial correlation structure for each assessment time, which may not hold in practice. **Aims.** This study aims to provide more flexible methods for modelling the spatio-temporal correlation in multi-assessment perennial crop data, allowing for differing spatial parameters for each time, together with modelling genetic effects over time. **Methods.** The paper investigates the suitability of two-directional invariant multivariate autoregressive (2DIMVAR1) models for analysis of multi-assessment perennial crop data. The analysis method is applied to persistence data from a pasture breeding trial. **Key results.** The multivariate autoregressive spatio-temporal residual models are a significant improvement on separable residual models under different genetic models. The paper demonstrates how to fit the models in practice using the software ASReml-R. **Conclusions.** A flexible modelling approach for multi-assessment perennial crop data is presented, allowing differing spatial correlation parameters for each time. The models allow investigation into genotype × time interactions, while optimally accounting for spatial and temporal correlation. **Implications.** The models provide improvements on current approaches and hence will result in more accurate genetic predictions in multi-assessment perennial crop variety selection trials.

Keywords: 2DIMVAR1, BLUP, genotype by environment interaction, linear mixed models, multivariate autoregressive model, perennial crop variety selection, random regression, spatial and temporal modelling, splines.

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Introduction

Perennial crop variety selection (in perennial pasture, grains, horticulture or forestry crops) is usually based on field trial evaluation, often in trials that exhibit spatial trends and correlation between plots. Selection is also usually based on measurements taken at multiple assessment (harvest or observation) times during the life of the crop. Thus, the genetic or variety effects may change over assessment times and these effects may be correlated. In addition, for the most accurate variety selections to be made, the statistical analysis must account for the spatial variation and correlation within a trial and the temporal correlation between repeated measurements on the same plot or plant. Furthermore, the spatial correlation between plots may vary between the multiple assessment times and this may need to be allowed for in the statistical analysis (De Faveri 2013).

The primary aim of these trials is selection of varieties. This usually involves treating the variety effects as random effects (Smith *et al.* 2005). The variety effects are likely to be related over time. There are two approaches to relating the repeated measurements on varieties over time. The first is to directly model the variance–covariance matrix of the

variety effects over time. The unstructured genetic covariance model may be used in some cases, whereas more parsimonious models such as the autoregressive or ante-dependence or factor analytic models (Smith *et al.* 2001) may be appropriate for modelling the genetic covariance structure for more observation times (Smith *et al.* 2007; De Faveri *et al.* 2015; Culvenor *et al.* 2017; Verbyla *et al.* 2021; Bally and De Faveri 2021).

The second approach is to propose a model for the (usually smooth) trend for varieties over time. This also results in a variance–covariance matrix for variety effects. Often the aim is to model the genetic response over time to allow for prediction at times other than assessment times and also to obtain a better insight into variety × time interactions (De Faveri *et al.* 2015). A suitable model for estimating the genetic response over time is the random regression (or random coefficients) model (Laird and Ware 1982).

Random regression models involve fitting regression coefficients on time (or other explanatory variables), for each variety, as random effects. This allows for variation between varieties in the shape of the response profile over time.

Random regression models may be implemented via orthogonal, Legendre or cubic polynomials (Campbell *et al.* 2018); however, they can also be implemented using more flexible bases such as splines, for example B splines (Meyer 2005) or cubic smoothing splines (Verbyla *et al.* 1999; White *et al.* 1999; Huisman *et al.* 2002; DeGroot *et al.* 2003). Verbyla *et al.* (1999) implemented cubic smoothing spline random regression for modelling the unit effects in order to account for the temporal correlation between repeated assessments. De Faveri *et al.* (2015) implemented linear random regressions with an underlying cubic smoothing spline for the mean response for persistence over time in a lucerne breeding trial. De Faveri *et al.* (2023) extended this approach to the multi-environment (MET) situation. Verbyla and Verbyla (2009) used the cubic smoothing spline in modelling of lactation curves for dairy cattle and then used the area under the curve in an association study.

Both approaches can provide a predictive model for variety effects over time. The first approach does so for each observed time and also other times if the variance–covariance model is 'smooth'; this is the approach used in geostatistics for instance where prediction across the spatial domain is important, for example using the Matérn function (Haskard *et al.* 2007).

Although the variety effects are the primary aim, they may be masked by non-genetic effects; these include effects due to the design of the trial and residual effects due to spatial and temporal variation. Hence, it is important to model these non-genetic effects effectively.

In the context of field trials for crops, Gilmour *et al.* (1997) introduced a spatial analysis approach for trials assessed at a single observation time, based on the linear mixed model that accounted for various sources of spatial variation, including local and global smooth trend and extraneous variation. In these models the local spatial correlation is typically modelled using a separable autoregressive process of order 1 in the row and column directions.

The literature showing the effectiveness and improvement of spatial modelling using the approach of Gilmour *et al.* (1997) in annual crops or forestry with a single measurement time is widespread (Costa e Silva *et al.* 2001; Dutkowski *et al.* 2002; Smith *et al.* 2005; Oakey *et al.* 2006; Welham *et al.* 2010).

In the case of perennial crops (e.g. perennial pasture crops; horticulture tree fruit and nut crops such as macadamia, apple, citrus and mango; other horticulture crops such as strawberry and pineapple; and forestry breeding with multiple measurements), there is the added complication of dealing with not only spatial correlation, but also spatially correlated repeated measurements over time.

When variety selection data are based on multiple assessment times, the statistical analysis methods need to account simultaneously for both spatial variation in the field and the temporal correlation between repeatedly measuring the same plot, plant or tree over time. The temporal correlation is likely to decrease with increasing time between assessments (Bjornsson 1978; Diggle 1988). The residual variance is also likely to vary over time. Not accounting for this spatial and temporal correlation and spatio-temporal interaction may result in biased variety estimates.

Optimal models for spatial and temporal modelling of longitudinal data in perennial crop variety trials are not widespread, and often simplistic models are implemented, such as simple repeatability models without spatial modelling (Smith *et al.* 1998, O'Connor *et al.* 2021), or univariate spatial modelling at individual timepoints, or longitudinal non-spatial modelling, for example using ante-dependence or spline models. De Resende *et al.* (2006) investigated and compared several of these different approaches for the spatial analysis of longitudinal data in perennial tea, showing the inadequacy of the simple repeatability model and the superiority of simultaneous longitudinal and spatial modelling. The full multivariate spatial model used by De Resende *et al.* (2006) was based on a linear mixed model with three-way (time by row by column) separable structured variance–covariance residual model. This approach was also implemented and extended in Smith *et al.* (2007), modelling sugarcane repeated measures variety selection data over two seasons, and De Faveri *et al.* (2015), modelling lucerne multi-harvest data over 10 timepoints. These separable residual models are an improvement on previous models; however, they are restrictive in that they assume common spatial parameters for each assessment time.

Although the separable models are likely to be better than not at accounting for any spatial correlation between plots, the separability assumption may not hold in some cases. Spatial correlation is likely to change over time, especially when perennial crops are measured over multiple seasons with varying environmental conditions such as rainfall or

temperature, and also as the plants or trees are changing in age, development stage and size.

De Faveri *et al.* (2017) introduced a more flexible residual variance–covariance model for analysis of multivariate data from field trials based on a multivariate autoregressive model of order 1. This model involves conditions to allow the process to be directionally invariant in the spatial dimensions. The resulting two-directional invariant multivariate autoregressive process of order 1 (2DIMVAR1) allows for differing spatial correlation parameters for each trait. De Faveri *et al.* (2017) applied the method to bivariate datasets in the combined analysis of yield and persistence at a single timepoint, where it outperformed the separable residual models in most cases.

The aim of this paper is to demonstrate the suitability and improvement of the 2DIMVAR1 residual model for modelling spatio-temporal correlation in the analysis of multi-assessment perennial crop data in conjunction with modelling of genetic effects over time. Although the 2DIMVAR1 model has been implemented for bivariate multi-trait data at a single timepoint, this paper is the first application for the analysis of repeated measurement data over multiple times. Its novelty lies in being able to model simultaneously spatial and temporal correlation, allowing for differing spatial correlation parameters for each measurement time. It is also the first application integrated with random regression spline genetic models for modelling genetic responses over time.

The method of analysis is applied to persistence data assessed over 5 years from a phalaris (*Phalaris aquatica* L.) perennial forage grass breeding trial. The models can be fitted using ASReml (Butler *et al.* 2017) in the R environment (R ver. 4.1.0; R Core Team 2021). A major aim of this paper is to provide code for the analyses to enable the implementation of the methods and this code is provided in the Supplementary material.

Materials and methods

Motivating data

The motivating dataset analysed in this paper comes from a perennial pasture grass variety selection trial conducted over 5 years (2009–13) by CSIRO (for complete details of the trial, see Culvenor *et al.* 2017). In this paper we investigate the analysis of a single site, namely B09, sown in 2009, near Beckom, NSW, Australia (34°15′59.65″S, 146°59′45.50″E; elevation 222 m a.m.s.l.; average annual rainfall 460 mm). The trial was conducted using a row–column design of eight rows by 15 columns, with four replicates and 30 varieties. Plot size was 4.5 m^2 (5 m by 0.9 m).

The varieties (lines) grown in the trial consisted of 29 phalaris lines and one cocksfoot (*Dactylis glomerata* subsp. *hispanica* L.) cv. Kasbah, the latter included as a persistent control (Culvenor *et al.* 2017). Variety ID numbers follow those in Culvenor *et al.* (2017) and are presented in Table 1.

Table 1. ID numbers for lines used in the study: line 25 is the control cocksfoot Kasbah and others are phalaris lines.

No.	Line
1	Northern retainer
2	Northern retainer MS
3	Northern
4	P × C
5	Sirocco retainer
6	CPI 19305
7	19305 Retainer
8	TamPWA F2
9	TamPWA F4
10	Sirocco
11	Perla Koleagrass
12	Atlas PG
13	Sirolan
14	Holdfast
15	Holdfast GT
16	Landmaster
17	Sirosa
18	Australian
19	Australian II
20	Accession CPI14697
21	Accession M91
22	Accession M170
23	Accession M196
24	Accession M225
25	Kasbah cocksfoot
26	Accession M241
27	Accession T39 selection
28	Accession S99
29	LD97
30	CPI 19315

The trait investigated in this paper is frequency of live plant base. Following the methodology of Lodge and Gleeson (1984) developed for measuring 'frequency' of lucerne stands, frequency was measured each winter in two 0.9 m^2 fixed quadrats by counting the number of 0.1 m by 0.1 m cells containing live phalaris base and converting to a percentage. Data were collected annually at five timepoints (2009–13). Changes in frequency during the experiment were used to assess persistence. The aim of the trial was variety selection based on persistence and relative to the Kasbah cocksfoot line.

A plot of the raw frequency data over time for each replicate of each variety is given in Fig. 1. The variety profiles are relatively smooth over time and most varieties follow a similar trend. There are differences between individual plot

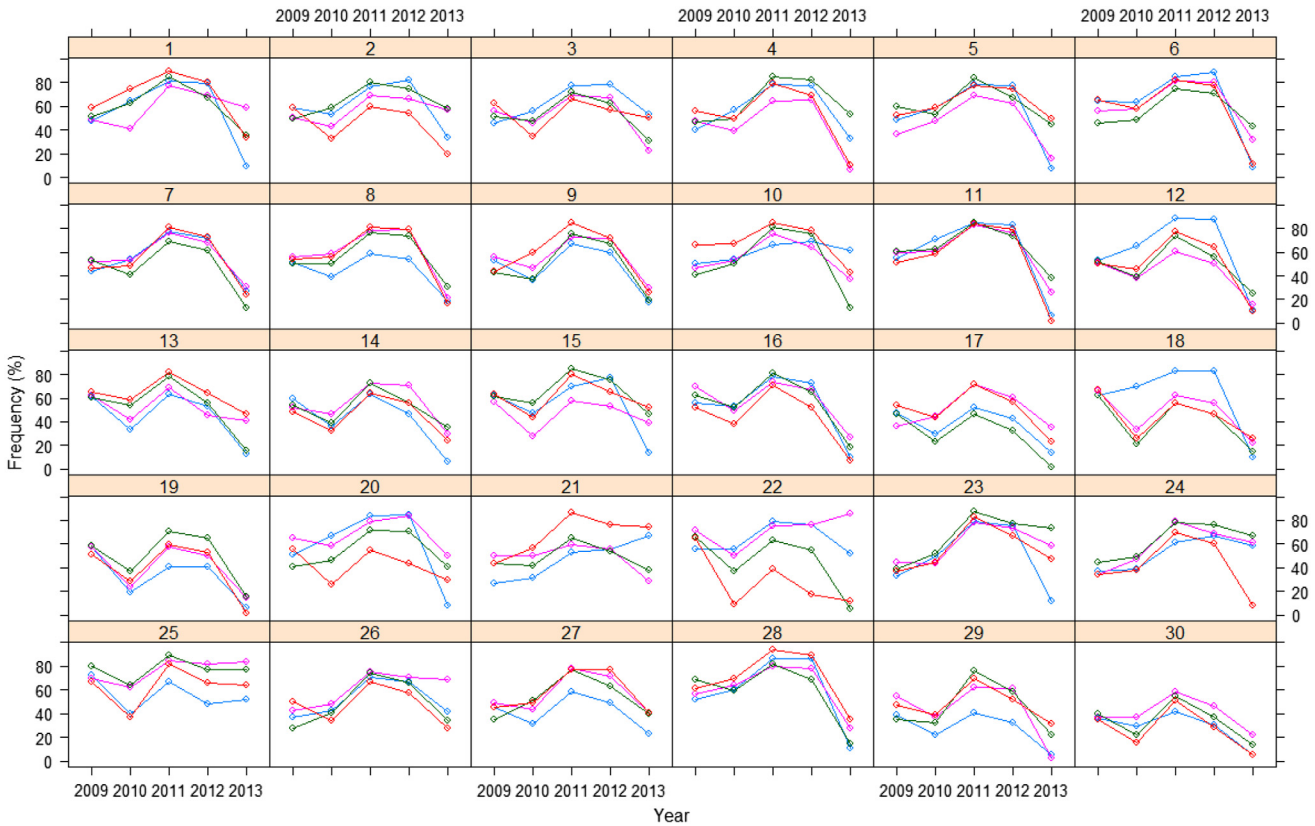

Fig. 1. Plot of raw frequency data for each variety over time (each line within a panel represents a replicate). Variety 25 is the Kasbah cocksfoot control.

responses within a variety, with some plots having a higher frequency response over the course of the trial and others consistently lower.

Statistical analyses

The phalaris frequency data was analysed using a multi-time analysis, similar to that in De Faveri *et al.* (2015) but with improved, novel spatio-temporal residual modelling. The variety effects were modelled over time accounting for any spatial and temporal correlation present. The analysis was based on a linear mixed model with estimation using residual maximum likelihood (REML). The analyses were performed in ASReml-R (Butler *et al.* 2017).

A linear mixed model for the data y (combined across assessment times) may be written as:

$$y = X\tau + Z_g u_g + Z_o u_o + e \quad (1)$$

where τ is a vector of fixed effects with design matrix X; u_g is a vector of random variety (or genetic) effects for t individual assessment-time combinations, with design matrix Z_g; u_o is a vector of other non-genetic random effects (e.g. replicate effects) with design matrix Z_o; and $\boldsymbol{e}$ is the vector of random residual effects.

The random effects from the linear mixed model are assumed to follow a normal distribution with zero mean vector and variance–covariance matrix:

$$\text{var}\left(\begin{bmatrix} u_g \\ u_o \\ e \end{bmatrix}\right) = \begin{bmatrix} G_g & 0 & 0 \\ 0 & G_o & 0 \\ 0 & 0 & R \end{bmatrix}$$

The variance model for the random non-genetic effects is given by a block diagonal matrix G_o. The variance matrix G_g for the genetic effects (u_g) across times may be represented by $G_g = G_h \otimes I_m$ where G_h is the genetic variance matrix indexed by the times, and I_m is the assumed structure for the varieties. Note that pedigree and/or genomic information could be included here, if available, to relate varieties via relationship matrices; see for example Oakey *et al.* (2006).

Modelling genetic effects

Unstructured or factor analytic model. The genetic variance matrix G_h (which consists of genetic variances for each assessment time and genetic co-variances between assessment time combinations) may be modelled using a variety of approaches including unstructured or factor analytic models (Smith *et al.* 2001, 2015). In Smith *et al.* (2007), G_h is

modelled using an unstructured (US) matrix, but they note that factor analytic (FA) models may also be suitable.

Random regression approach. An alternative approach for modelling the genetic effects over time is to use a random regression model. In this approach, the genotype deviations from the underlying mean trend over time are modelled. Often whilst the underlying mean trend may be non-linear, the genotype deviations may be linear over time (Evans and Roberts 1979), but they may vary over varieties. Because we are interested in selection of varieties, the intercept and slope of these deviation responses may be taken as random rather than fixed, similar to the basic quantitative genetics model in which genotype effects are taken as a random factor. If the genetic effects u_g have components $u_{g,ik}$ for genotype i, time k ($k = 1,\ldots,t$), where the actual time is x_k, a linear random regression model is:

$$u_{g,ik} = u_{i0} + u_{i1}x_k + u_{e,ik} \tag{2}$$

where u_{i0} and u_{i1} are the random intercept and slopes for genotype i, and $u_{e,ik}$ is a residual deviation from the linear random regression, that is, a lack-of-fit term. The intercepts u_{i0} provide a prediction of performance at $x_k = 0$, so typically the time variable is centred, so that the origin is at the midpoint, or average of the times. The slopes u_{i1} provide the rate of change of the effect of the genotype, and hence the speed at which the performance changes over time.

This linear random regression model (2) may be extended to a polynomial random regression model to model non-linear trends, or alternatively, it may be preferable to use natural cubic splines (Verbyla *et al.* 1999) to provide a more flexible approach (De Faveri *et al.* 2015, 2023).

A random regression model incorporating cubic smoothing splines, for $u_{g,ik}$ (the random effect for variety i at assessment k ($k = 1,\ldots,t$)) with x_k denoting the time at assessment k, can be written as:

$$u_{g,ik} = u_{i0} + u_{i1}x_k + \boldsymbol{z}_{\boldsymbol{sk}}^T\boldsymbol{u}_{\boldsymbol{si}} + u_{e,ik} \tag{3}$$

For each variety i, $\boldsymbol{u}_{\boldsymbol{si}}$ (a $(t-2)\times 1$ vector) is the random spline component of the mixed model formulation of the cubic smoothing spline (Verbyla *et al.* 1999).

Modelling non-genetic effects

The residual covariance matrix R models the spatial correlation and temporal correlation between repeated measurements. This residual covariance matrix has been modelled in this paper using two approaches, including:

1. A three-way separable spatio-temporal process (De Resende *et al.* 2006; Smith *et al.* 2007; De Faveri *et al.* 2015). Therefore, the structure is assumed to be:

$$\boldsymbol{R} = \boldsymbol{R}_h \otimes \sum\nolimits_c \otimes \sum\nolimits_r$$

where $\boldsymbol{R}_h$ is a covariance matrix that incorporates temporal correlation (between assessment times) and, possibly, heterogeneous variance across times, and $\boldsymbol{\Sigma}_c$ and $\boldsymbol{\Sigma}_r$ are the column and row local spatial correlation matrices, here taken as autoregressive models of order 1 (ar1) with spatial correlation parameters ϕ_r and ϕ_c in the row and column directions, respectively (Gilmour *et al.* 1997). In the analyses, the temporal covariance components ($\boldsymbol{R}_h$) have been modelled using unstructured, heterogeneous autoregressive and ante-dependence models (Gabriel 1962). In these analyses, the separable residual models assume the same spatial correlation parameters (ϕ_r and ϕ_c) for each assessment time.

2. A two-directional invariant multivariate autoregressive of order 1 (2DIMVAR1) model (De Faveri *et al.* 2017), where e_{ij}, the multivariate error variable for row i and column j for t times is given by:

$$\boldsymbol{e}_{11} = \boldsymbol{\epsilon}_{11}$$

$$\boldsymbol{e}_{i1} = \boldsymbol{\Omega}_r\boldsymbol{e}_{(i-1)1} + \boldsymbol{\epsilon}_{i1}, \qquad i = 2,3,\ldots,r$$

$$\boldsymbol{e}_{1j} = \boldsymbol{\Omega}_c\boldsymbol{e}_{1(j-1)} + \boldsymbol{\epsilon}_{1j}, \qquad j = 2,3,\ldots,c$$

$$\boldsymbol{e}_{ij} = \boldsymbol{\Omega}_r\boldsymbol{e}_{(i-1)j} + \boldsymbol{\Omega}_c\boldsymbol{e}_{i(j-1)} + \boldsymbol{\Omega}_{rc}\boldsymbol{e}_{(i-1)(j-1)} + \boldsymbol{\epsilon}_{ij},$$
$$i = 2,3,\ldots,r;\ j = 2,3,\ldots,c$$

where $\boldsymbol{\epsilon}$ has zero-mean vector and $\boldsymbol{\epsilon}_{ij}$ are mutually independent vector variates, $\mathrm{var}(\boldsymbol{e}_{ij}) = \boldsymbol{\Sigma}$, for all i and j, and $\boldsymbol{\Omega}_r$ and $\boldsymbol{\Omega}_c$ are $t \times t$ matrices of spatial dependence parameters in the row and column direction, respectively.

It can be shown that under this model, $\boldsymbol{R}$ can be written as the sum of t terms, each the Kronecker product of two autoregressive models and a reduced rank factor analytic model:

$$R = \sum_{s=1}^{t} \begin{bmatrix} 1 & \phi_{cs} & \ldots & \phi_{cs}^{c-1} \\ \phi_{cs} & 1 & \ddots & \vdots \\ \vdots & \ddots & \ddots & \phi_{cs} \\ \phi_{cs}^{c-1} & \phi_{cs}^{c-2} & \ldots & 1 \end{bmatrix} \otimes \begin{bmatrix} 1 & \phi_{rs} & \ldots & \phi_{rs}^{r-1} \\ \phi_{rs} & 1 & \ddots & \vdots \\ \vdots & \ddots & \ddots & \phi_{rs} \\ \phi_{rs}^{r-1} & \phi_{rs}^{r-2} & \ldots & 1 \end{bmatrix} \otimes p^s p^{sT}$$

This model allows for different spatial correlation parameters for each assessment time.

Full details of the 2DIMVAR1 model can be found in De Faveri *et al.* (2017); in summary, the model fits a multivariate analogue of the first-order autoregressive spatial model in row and column directions $(ar1(Col) \otimes ar1(Row))$ across times (t), with the spatial correlation parameters (ϕ_r and ϕ_c) replaced by $t \times t$ spatial matrices $\boldsymbol{\Omega}_r$ and $\boldsymbol{\Omega}_c$.

The diagonal elements of $\boldsymbol{\Omega}_r$ give the spatial dependency parameters between neighbouring plots in the row direction at each time, while the off diagonals give the spatial dependency between neighbouring plots at different times, hence allowing for differing spatial correlation across the times (similarly for $\boldsymbol{\Omega}_c$). These spatial dependency matrices are not correlation matrices and do not need to be symmetric. Together with a fully unstructured covariance matrix $\boldsymbol{\Sigma}$ (which models the temporal variance and covariances between times on each plot), the residual spatio-temporal process is modelled. Symmetry constraints on $\boldsymbol{\Omega}_r\boldsymbol{\Sigma}$ and $\boldsymbol{\Omega}_c\boldsymbol{\Sigma}$ (the spatio-temporal covariance matrices between neighbouring plots in the row and column directions, respectively) make the model directionally invariant in the row and column directions, so the full variance–covariance matrix is the same whether observations are ordered from left to right or from right to left along the rows or columns.

To test the significance of random effects in the linear mixed model, the residual maximum likelihood ratio test (REMLRT) can be used. The REMLRT may be used to compare the fit of two models only if they are nested and contain the same fixed effects. The standard REMLRT statistic is asymptotically distributed as a chi-squared statistic with $p_1 - p_0$ degrees of freedom. However, if the test involves a null hypothesis where the parameter is on the boundary of the parameter space, the REMLRT needs to be adjusted (see Stram and Lee 1994).

To compare the goodness of fit of two models (with the same fixed effects) that may be non-nested, the Akaike information criterion (AIC) may be used. The AIC value for a model is calculated as $-2(l - p)$, where l is the residual log-likelihood for the model and p is the number of variance parameters in the model. To compare models with different fixed effects, an AIC based on the full likelihood may be used (Verbyla 2019). Models with smaller AIC values provide a better fit to the data than those with higher AIC values.

Results

The analysis of frequency was based on the linear mixed model given in Eqn 1. A series of genetic and residual models was fitted to the data and results are presented in Table 2. In each analysis, the random experimental design terms for Rep, Row and Column for each year were included in the model.

Initially a separate analysis at each time was conducted with a non-spatial model (M1). The residuals from this model are presented in Fig. 2, where it can be seen that the spatial pattern of residuals differs between assessment times. In addition to accounting for the trial design, the next model (M2) followed the spatial analysis approach of Gilmour *et al.* (1997) and Stefanova *et al.* (2009), including terms for extraneous and global trend and modelling local spatial correlation using a separable autoregressive process of order 1 in the row and column directions.

Comparing results between model M1 and model M2 clearly shows that accounting for the spatial correlation between plots results in a significant improvement ($P < 0.001$; REMLRT = 81.264 on 10 d.f.). This initial spatial analysis provides insight into the spatial correlation at each Year. The spatial correlation parameters and residual variances estimated for each assessment time from the initial single time analyses are given in Table 3. From these results, the

Table 2. Summary of models fitted to the multi-assessment data with genetic model (G), residual model (R), other non-genetic random terms, residual log-likelihoods (LL), and Akaike information criteria (AIC) values (based on full likelihood) for each Model.

Model	Genetic model (G)	Residual model (R)	Other random terms	LL	AIC
M1	Diag(Year):Line	id(Col) × id(Row) | Year	At(Year): (Rep + Row + Col)	−1741.564	3545.429
M2	Diag(Year):Line	ar1(Col):ar1(Row)| Year	At(Year): (Rep + Row + Col)	−1700.932	3479.507
M3	Diag(Year):Line	ar1(Col):ar1(Row)| Year	At(Year): (Rep + Row + Col) + Plot	−1603.266	3274.839
M4	Diag(Year):Line	diag(Year):ar1(Col):ar1(Row)	At(Year): (Rep + Row + Col) + Plot	−1612.799	3283.941
M5	US(Year):Line	ar1h(Year):ar1(Col):ar1(Row)	At(Year): (Rep + Row + Col) + Plot	−1585.288	3245.676
M6	US(Year):Line	ante(Year,1):ar1(Col):ar1(Row)	At(Year): (Rep + Row + Col) + Plot	−1545.252	3173.160
M7	US(Year):Line	corgh(Year):ar1(Col):ar1(Row)	At(Year): (Rep + Row + Col) + Plot	−1542.028	3175.831
M8	**US(Year):Line**	**2DIMVAR1**	At(Year): (Rep + Row + Col)	**−1526.688**	**3115.341**
M9	RR	ar1h(Year):ar1(Col):ar1(Row)	At(Year): (Rep + Row + Col) + Plot	−1631.308	3314.643
M10	RR	ante(Year,1):ar1(Col):ar1(Row)	At(Year): (Rep + Row + Col) + Plot	−1580.344	3220.703
M11	RR	corgh(Year):ar1(Col):ar1(Row)	At(Year): (Rep + Row + Col) + Plot	−1576.359	3220.761
M12	**RR**	**2DIMVAR1**	At(Year): (Rep + Row + Col)	**−1547.583**	**3173.185**

Models M1–M4 fit separate genetic effects for each assessment time (Year), whereas models M5–M8 fit a fully unstructured covariance model for the genetic effects across times and models M9–M12 fit a random regression (RR) model for genetic effects over time: Line + lin(time):Line + spl(time):Line + lack of fit term dev(time):Line. In models M1–M8 a separate fixed effect mean for each assessment time (Year) has been fitted, whereas in models M9–M12 the overall mean has been modelled over time using the spline model: 1 + lin(time) + spl(time) + lack of fit term dev(time). Model terms are detailed in the Supplementary material.

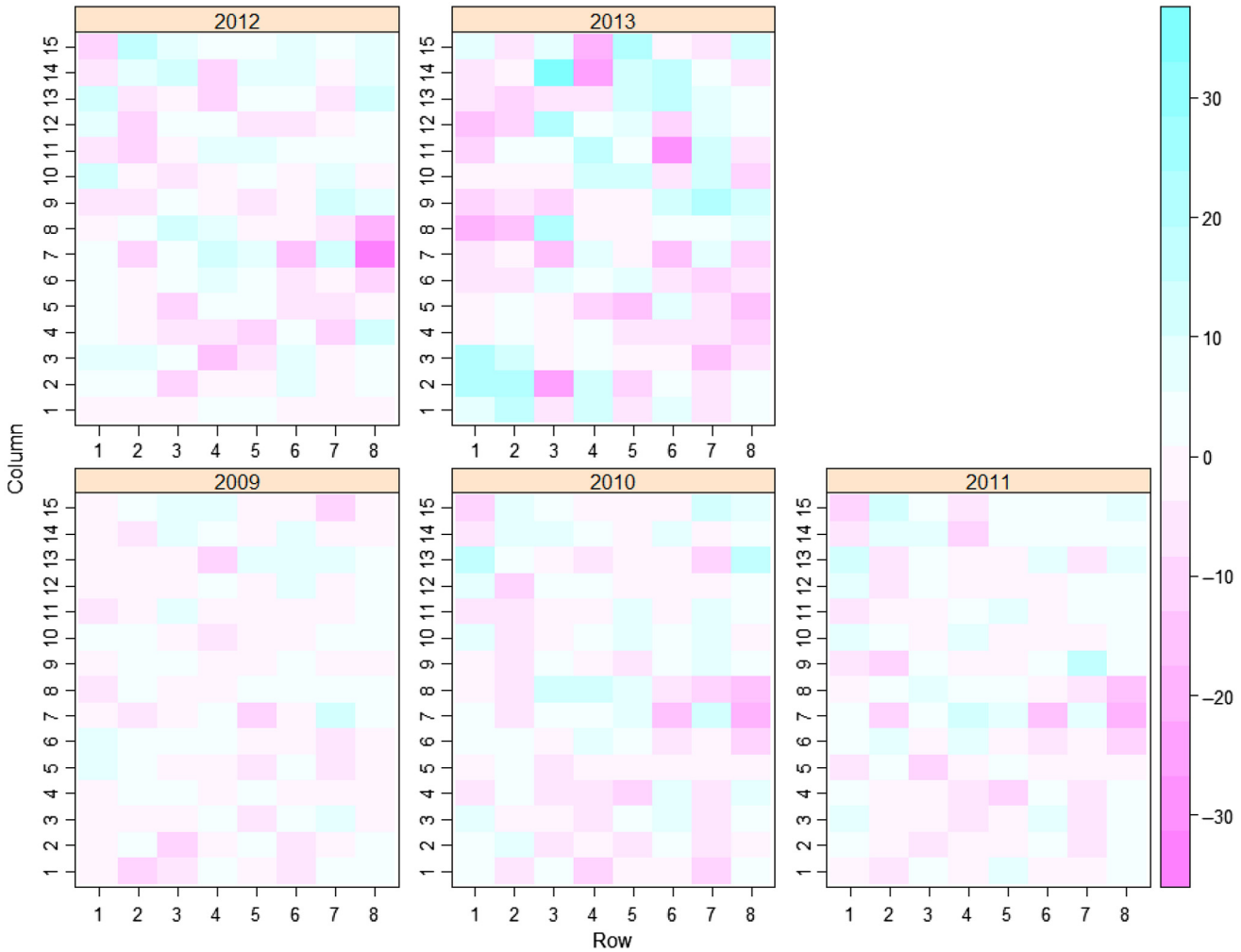

Fig. 2. Plot of residuals for each year from model M1 (id(Col) × id(Row)).

Table 3. Residual variances and spatial correlation parameters for each year from individual analyses of frequency data at each time (M2).

Year	Residual variance	Column spatial parameter ϕ_c	Row spatial parameter ϕ_r
2009	37.364	0.261	0.385
2010	84.575	0.528	−0.048
2011	67.568	0.562	−0.118
2012	106.915	0.582	0.046
2013	261.859	0.622	0.049

residual variance can be seen to increase over time. The spatial correlation parameters can also be seen to vary across years with ϕ_c ranging from 0.261 to 0.622 and ϕ_r ranging from −0.118 to 0.385. This difference in spatial parameters across times indicates that there may be a need to allow for flexible modelling of the spatial parameters over time.

The next model (M3) fitted an overall Plot term, which was significant (REMLRT). The following model (M4) fitted a separable spatial and temporal model allowing for different residual variances for each time but did not model the correlation between times. This separable residual model assumed common row and column spatial parameters across all times, with the common spatial correlation parameters estimated as (ϕ_c = 0.400, and ϕ_r = 0.067). This model was not a significant improvement over M3, which allowed for separate spatial parameters across times, indicating that the separable residual models may not be ideal in this situation.

Subsequent models fitted more suitable genetic covariance models to the genetic effects over time, correlating the genetic effects over time.

Models 5, 6 and 7 fitted an unstructured covariance structure to the genetic effects over time, together with different residual models. In this trial the number of measurement times was low (five times), so it was possible to fit the unstructured genetic model. In situations of more measurement times, the number of parameters requiring estimation in the unstructured model is likely to be prohibitive and more parsimonious models are desired (e.g. factor analytic (FA)

models). In these three unstructured genetic models, the residual effects have been modelled using a three-way separable spatial and temporal residual model (similar to M4), but in these models the temporal covariance structure is modelled firstly using a heterogeneous autoregressive model of order1 (ar1h) (M5), then an ante-dependence model of order 1 (M6), and then a fully parameterised unstructured model (M7). Based on AIC values, the ante-dependence model was best out of these separable residual models.

The next model (M8) incorporated the multivariate extension of the $ar1(Col) \otimes ar1(Row)$ spatial model, the directionally invariant 2DIMVAR1 residual model, once again with an unstructured genetic model. This residual model allows for differing spatial parameters for each time, hence providing a more flexible spatial and temporal structure. The 2DIMVAR1 model was a significant improvement on the other residual models (REMLRT $P < 0.001$ in each case).

The final three models fitted a more parsimonious structure to the genetic effects over time using a random regression approach. This approach models the smooth trend across time, allowing for predictions at times other than the measurement times. The models include an underlying overall mean level cubic smoothing spline across times, and random variety deviations are modelled (also using cubic smoothing splines) about this overall mean response, as in Eqn 3. The random variety effects (intercepts and slopes) have been correlated to make the model invariant to translation. It would also be desirable to correlate the random spline components with the random intercepts and slopes to make the model invariant to a change in basis (Fitzmaurice *et al.* 2009), but this correlation was unable to be fitted. Once again in this set of models, the model with the non-separable 2DIMVAR1 residual model was a significant improvement on the models with separable autoregressive (M9), ante-dependence

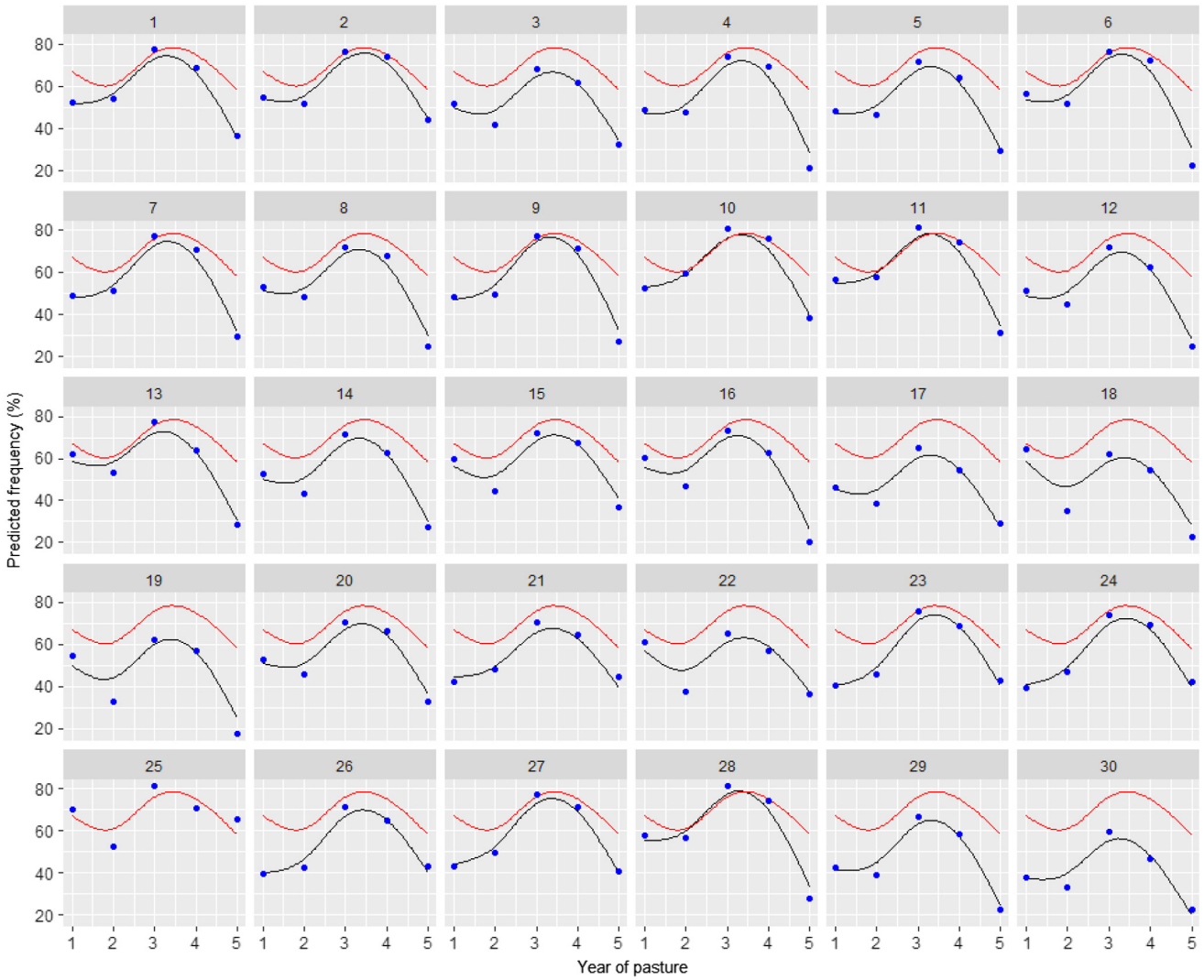

Fig. 3. Plot of predictions for each variety (black line) and control (25) (red line) from the random regression model with 2DIMVAR1 residual model (M12) together with predictions at each assessment time (blue points) from the unstructured genetic model with 2DIMVAR1 residual model (M8).

(M10) and unstructured (M11) by $ar1(Col) \otimes ar1(Row)$, residual models (REMLRT $P < 0.001$ in each case).

Variety predictions over time have been made from the best random regression model (M12) and smooth responses for each variety plotted in Fig. 3. Predictions from the best unstructured genetic model (M8) have also been included in this plot. Both of these models included the 2DIMVAR1 residual model. In this figure, the control variety (25) response is also plotted in each panel for comparison. The varieties all follow a similar trend, so to compare varieties, it is more informative to investigate the variety deviations from the underlying mean trend. These variety deviations (from M12) are presented in Figs 4 and 5.

There is considerable variety × year interaction as seen in the crossovers between deviation curves in Fig. 5. The persistent control (25) is high in persistence throughout the trial and especially at the start and end, when it is higher than all other varieties. Some varieties are consistently low (e.g. 30) whereas some start low and continually increase relative to the mean (e.g. 26 and 27). Some varieties do better in the middle years (e.g. 28, 11, 10) and some do better at the end (e.g. 2). Some start high and then decrease (e.g. 18, 16, 13).

As an overall measure of frequency to provide a single comparison measure to rank varieties over time, the area under each predicted variety response curve (from M12) has been calculated. A plot of this total area under curve (AUC) versus final frequency is presented in Fig. 6, giving an insight into those varieties with overall high frequency and high final counts at the end of the trial.

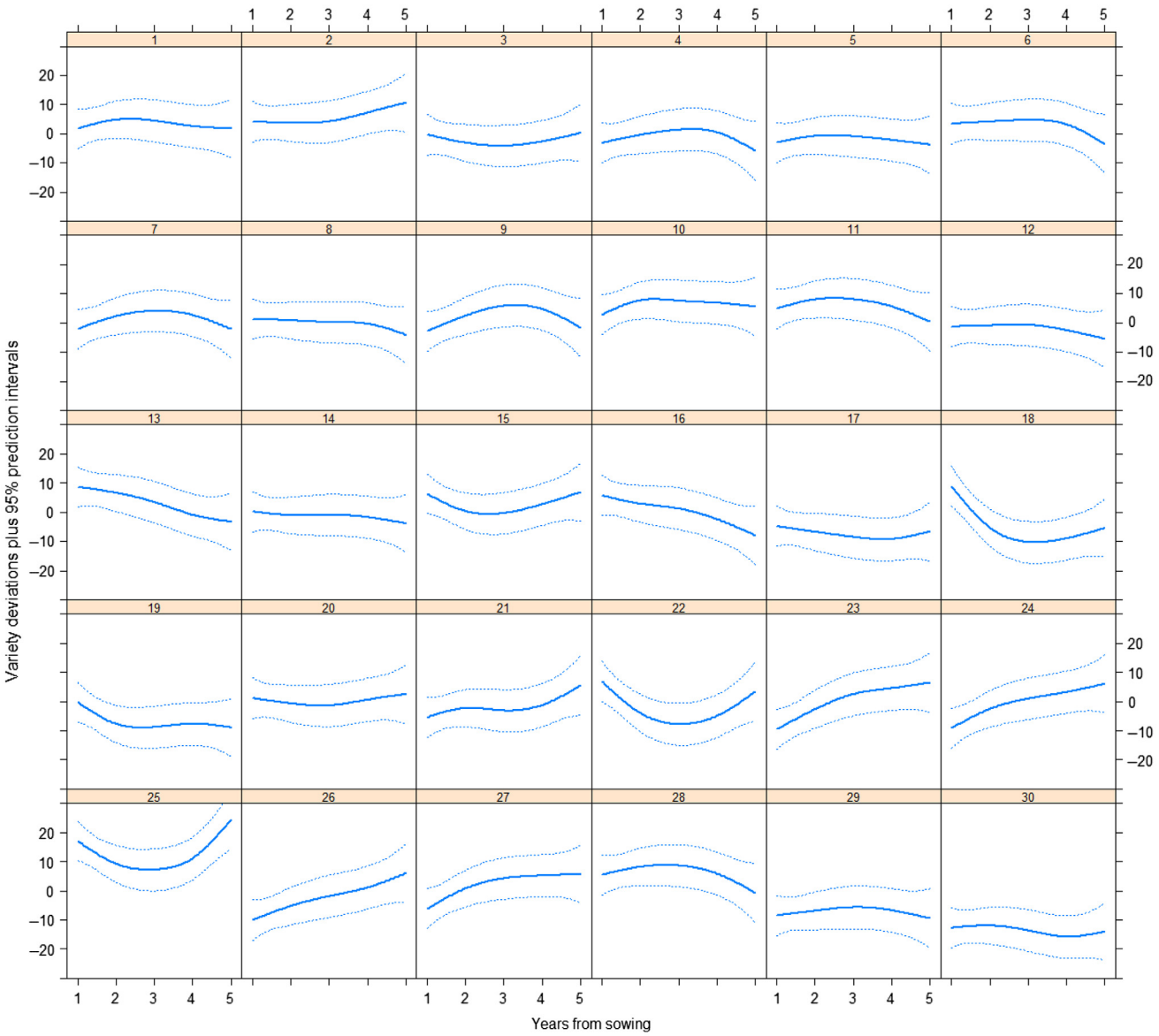

Fig. 4. Plot of variety deviations from overall mean response over time from model M12 together with 95% prediction intervals.

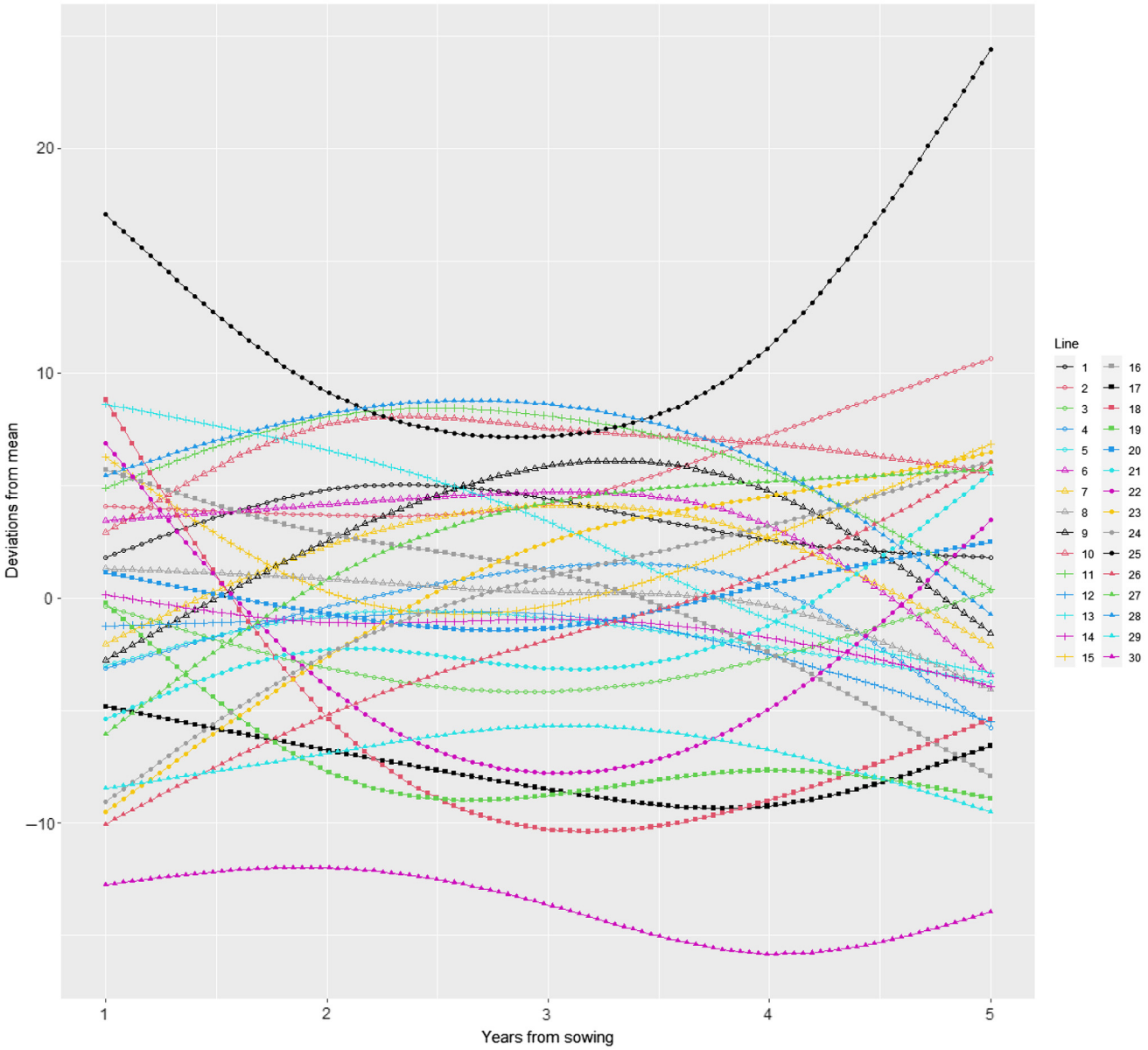

Fig. 5. Plot of variety deviations from overall mean response over time from model M12.

The control variety (25) can be seen to have the greatest AUC and final frequency, while variety 30 has very low AUC and final frequency. Varieties 2 and 10 have high AUC and also high final frequency, whereas varieties 11 and 28 have high AUC but slightly lower final frequency.

To provide insight into how the choice of residual model may affect the variety predictions, the best linear unbiased predictions (BLUPs) from models M1 (separate non-spatial analysis at each time), M5 and M8 (US genetic model with 3-way separable $ar1h(time) \otimes ar1(Col) \otimes ar1(Row)$ and 2DIMVAR1 residual model, respectively), and M9 and M12 (CSS random regression genetic model with 3-way separable $ar1h(time) \otimes ar1(Col) \otimes ar1(Row)$ and 2DIMVAR1 residual model, respectively) have been plotted together in Fig. 7. There are clear differences in the genetic effects depending on the residual model fitted.

Predicted residual spatial dependency matrices and spatio-temporal correlations from the final model (M12) are presented in the Supplementary material.

Discussion

The analyses presented in this paper of frequency from a perennial pasture variety trial have shown the 2DIMVAR1 residual model to be superior to all of the separable spatial and temporal models fitted under a selection of different genetic models. The models have been compared using AIC

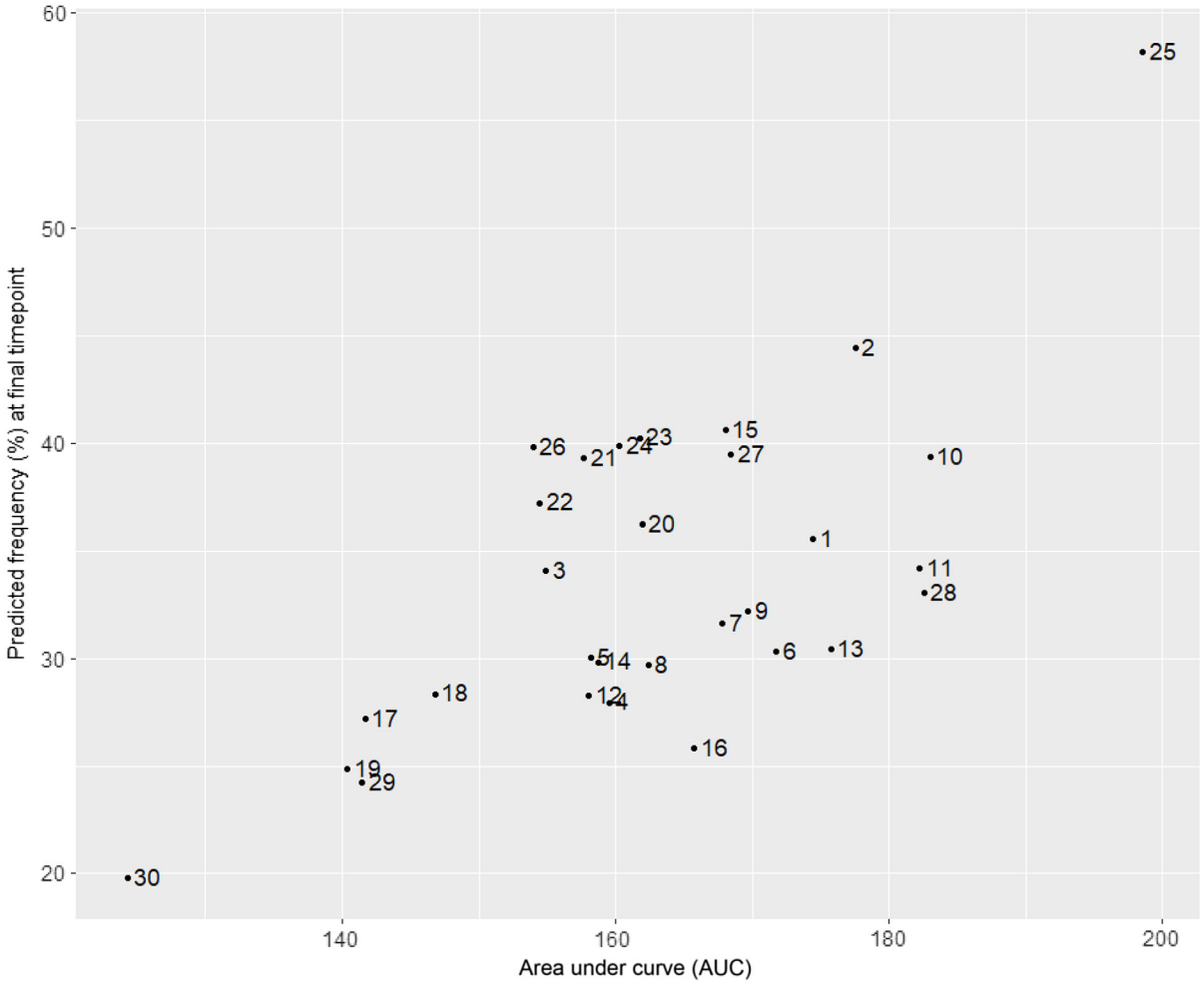

Fig. 6. Plot showing the area under the curve versus predicted final frequency for each variety predicted from model M12.

values based on the full likelihood (Verbyla 2019). The impact of the different residual models on prediction of genetic effects has been shown in plots of the BLUPs from competing models.

The 2DMVAR1 models were fitted in the software package ASReml-R, taking only seconds per iteration. As the number of measurement times increases, the number of parameters required for estimation in the 2DIMVAR1 model also increases, thereby increasing the computation time and decreasing the feasibility of fitting the 2DMVAR1 model in this form for large numbers of times. It is estimated that any more than 10–12 measurement times may cause computational difficulties using this formulation. Alternative, more parsimonious forms of the 2DIMVAR1 model may be possible (De Faveri 2013) but at this time are unable to be implemented in ASReml-R. This is an area of future research.

The flexibility provided by the 2DIMVAR1 residual model makes sense biologically by allowing for differing spatial correlation at each measurement time. It would be expected that local spatial correlation may be impacted by factors such as soil moisture levels that may vary over time and stage of growth of the plants. The current approach of modelling the spatial and temporal correlation using separable residual models that assume common spatial parameters over time is likely to be restrictive. In many cases, the extra flexibility provided by the 2DIMVAR1 model will provide a statistically better model.

The 2DIMVAR1 residual models in conjunction with random regression genetic models using cubic smoothing splines are easily implemented in the linear mixed model framework, providing an efficient approach for modelling variety profiles over time. The models allow overall rankings to be made across times (for overall performance) and also provide an approach to investigate genotype × time interactions. The models have been implemented here assuming independence between varieties but can easily be extended to include pedigree or genomic relationship information between varieties. The approach can also be extended to the multi-environment situation so that genotype × environment × time interactions may be investigated.

Although the random regression genetic models did not fit as well (based on AIC) as the fully unstructured genetic models in this example (this may be a result of very few

Fig. 7. Plot of variety BLUPs for each year (represented by colour) from models M1 (separate analysis at each time), M5 and M8 (US genetic model with 3-way separable and 2DIMVAR1 residual model, respectively), and M9 and M12 (CSS random regression genetic model with 3-way separable and 2DIMVAR1 residual model, respectively).

measurement times), they enabled in-depth investigation into variety × time interactions and predictions between assessment times. With higher numbers of measurement times, the unstructured model will require too many parameters to be estimated, and the benefit of the more parsimonious genetic models such as the random regression approach is likely to be more evident.

Conclusion

Spatial and temporal correlation affects the prediction of genetic effects over time from perennial pasture field trials, so it is important to model this correlation appropriately. The 2DIMVAR1 residual model provides a flexible covariance structure for modelling this spatial and temporal correlation, allowing for differing spatial correlation across measurement times. This model has been shown to be a significant improvement on more traditional separable spatio-temporal models and, together with parsimonious random regression genetic models, enables investigation into variety × time interactions in perennial crop evaluation.

Supplementary material

Supplementary material is available online.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author. R functions for fitting the 2DMVAR1 models are available from the author on request.

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