Theor Appl Genet (1993) 86:349–355



# Bias in genetic variance estimates due to spatial autocorrelation

**S. Magnussen**

Genetics of Growth and Yield, Forestry Canada, Petawawa National Forestry Institute, Chalk River, Ontario, Canada K0J 1J0

Received May 7, 1992; Accepted September 19, 1992
Communicated by A. R. Hallauer

**Summary.** A central problem in the analysis of genetic field trials is the dichotomy of "genetic" and "environmental" effects because one cannot be defined without the other. Results from 768,000 simulated family trials in complete randomized block designs demonstrated a serious upward bias in estimates of family variance components from multi-unit plot designs when the phenotypic observations were compatible with a first-order autoregressive (AR1) process. The inflation of family variances and, thus, additive genetic variance and narrow sense individual heritabilities progressed exponentially with an increase in the nearest neighbor correlation ($\varrho$) in the AR1 process. Significant differences in inflation rates persisted among various plot configurations. At $\varrho = 0.2$ the inflation of family variances reached 48–73%. Inflation rates were independent of the level of heritability. Modified Papadakis nearest-neighbor (NN) adjustment procedures were tested for their ability to remove the bias in family variances. A NN-adjustment based on Mead's coefficient of inter-plant interaction and one derived from Bartlett's simultaneous autoregressive scheme removed up to 97% of the bias introduced by the phenotypic correlations. NN-adjusted estimates had slightly (5–8%) higher relative errors than did unadjusted estimates.

**Key words:** Genetic variance – Experimental design – Simulation – Spatial process – Nearest neighbor adjustment

## Introduction

Nearest-neighbors (NN) in field trials share a common microsite that tends to make observations from non-competing neighbors more alike than trial observations selected at random (Cliff and Ord 1981; Upton and Fingleton 1985). This lack of independence among neighboring experimental units is known to cause bias in estimated treatment effects and in the standard error of treatment contrasts (Binns 1987; Kempton and Howes 1981; Magnussen 1990; Wilkinson et al. 1983); it also inflates the among-plot variance (Magnussen 1989a; Smith 1938; Snedecor and Cochran 1971). Today the analyst of field trials enjoys a plethora of spatial methods and NN-adjustment techniques that may improve both the precision and the accuracy of field experiments (for example, Correll and Anderson 1983; Cullis and Gleeson 1989; Ord 1975; Stein and Corsten 1991; Zimmerman and Harville 1991). Despite this progress and the suspicion that conventional analyses of spatially correlated observations in genetics trials may be disastrous (Stroup and Mulitze 1991) little has been undertaken to quantify, in a systematic way, the effect of NN-correlated observations on estimates of genetic variances.

A major obstacle to progress in this area is rooted in the confounding of genetic and environmental effects in any given trial (Gregorius and Namkoong 1987). An estimated "genetic effect" is conditional on a reference population and a reference environment and vice versa (Monserud and Rehfeldt 1990). Faced with a positive correlation among first-order neighbours in a field trial the question of how to decompose the phenotypic observation into orthogonal and unbiased "genetic" and "environmental" effects becomes intricate. The study presented here seeks to establish, through simulations, the impact of a phenotypic first-order autoregressive (AR1) process on estimates of family variance components in complete randomized block designs. Empirical correlation patterns appear to be compatible with this type of process (Besag and Kempton 1986; Binns 1987; Magnussen 1990). Designs included in the simulations are

those most commonly encountered in forest genetics trials where the problem of spatial dependencies is especially bothersome due to the large number of entries, patchy microsite mosaics, and the long duration of testing that allows the emergence of manifest spatial interrelationships (Correll and Cellier 1987; Libby and Cockerham 1980; Stern 1965). A choice of feasible (not necessarily optimal) NN-adjustment procedures will be introduced and assessed on their ability to remove bias in family variance components arising from correlations of phenotypic values.

## Methods

Simulated family trials in complete randomized block designs with *a priori* known family effects and AR1 [1] correlated phenotypic values were analyzed using conventional ANOVA procedures in order to assess the effects of the environmental spatial correlation on the estimated family variance component. Subsequently, nearest neighbor (NN) adjustments procedures demonstrated their potential for removing bias in family variance components introduced by the AR1-driven phenotypic correlations.

Each simulation began with the layout of a field of rows and columns consistent with a given experimental design. The field of experimental units was then divided into $n_{rep}$ contiguous blocks, each containing one plot of size L × W for each of the $n_{fam}$ families and families assigned randomly to plots (via random permutations). In a single simulation all experimental units belonging to a certain family (i) had the same family value $\tau_i$ assigned to it by a random draw from a Gaussian population with mean zero and variance $\sigma^2_{fam}$. Phenotypic values ($U$) associated with the experimental unit in location ($x$, $y$) came from the following generating algorithm:

$$U_{x,y} = \varrho \times U_{x-1,y} + \varrho \times U_{x,y-1} - \varrho^2 \times U_{x-1,y-1} + Z_{x,y} + \tau_i \quad (1)$$

where $\varrho$ denotes the spatial autocorrelation $|\varrho| < 1$ (row correlation is equal to column correlation), and $Z_{x,y}$ is an *iid* Gaussian residual environmental effect with mean zero and a variance of $\sigma^2(1-\varrho^2)^2$, where $\sigma^2$ is the total environmental variance ($\sigma^2 = 1$); $\tau_i$ is the *a priori* known effect of family $i$ assigned (randomly) to the location ($x$, $y$). For a process generated this way, the covariance between two non-related experimental units $r$ rows and $s$ columns apart is $\varrho^{(r+s)}$, as expected for an AR1 process (Brockwell and Davis 1987). To initiate this generating algorithm a single surround row ($x=0$) and column ($y=0$) of uncorrelated random Gaussian numbers with mean zero and a variance of 1.0 was created first before the experimental field was simulated. Note that the algorithm in Eq. (1) generates both "genetic" and "environmental" correlations as part of $\varrho$ and that the "genetic" component of the correlation will be design dependent. Intuitively, this may seem implausible in the light of the classical "Phenotype = Genotype + Environment" equation applied in the conventional analytical framework (Falconer 1981). However, this orthogonal decomposition requires a strict independence between the factors which, of course, is lacking in real life situations. The generation algorithm is merely a tool to create a field where adjacent phenotypes are more similar than phenotypes picked at random and where, by definition, the genetic values are confounded with the environmental values (i.e., a single observation includes a genotype × environment interaction effect). An example of a simulated field trial is provided in Fig. 1. The suggested model is, according to extensive analyses of field data (Falconer 1981), not only realistic but also closely models the real world.

[1] First-order autoregressive process

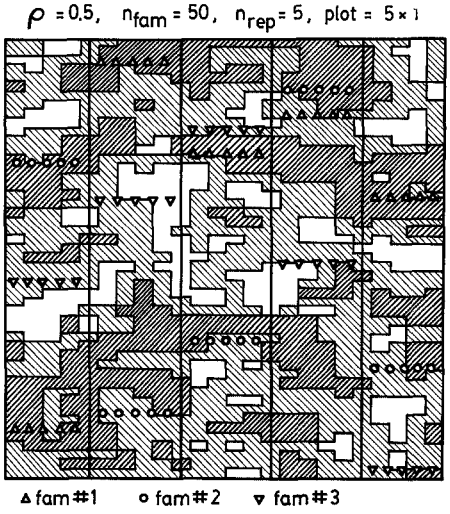


**Fig. 1.** An example of a simulated field trial with different signatures for high, medium and low phenotypic values. The locations of three families have been highlighted

A standard ANOVA of the simulated observations $U_{x,y}$ was performed on all simulated trials according to the model:

$$U_{x,y} = U_{ijk} = \mu + \tau_i + \beta_j + \pi_{ij} + \varepsilon_{ijk}, \quad (2)$$

where $U_{ijk}$ is the phenotypic observation made on the experimental unit $k$ in block $j$ belonging to family $i$, $\mu$ is the overall mean of all experimental units ($E(\mu) = 0$), $\tau_i$ is the family effect of family $i$ ($E(\tau)=0$, $E(\tau^2)=\sigma^2_f$), $\beta_j$ is the random effect associated with block $j$ ($E(\beta)=0$, $E(\beta^2)=\sigma^2_{rep}$), $\pi_{ij}$ is the effect of the plot associated with family $i$ in block $j$ ($E(\pi_{ij})=0$, $E(\pi^2)=\sigma^2_{plot}$) and $\varepsilon_{ijk}$ is the residual (error term) associated with the observation $U_{ijk}$ ($E(\varepsilon)=0$, $E(\varepsilon^2)=\sigma^2_w$). Variance components for families, replications, plots and within-plots were estimated by equating ANOVA mean-squares (MS) to their expectations (EMS). Table 1 outlines the form of the ANOVA. $F$-ratios to test the null hypotheses of $\hat{\sigma}^2_{fam}=0$ were computed as $F_{fam} = MS_{fam}/MS_p$, and the probability $P(F > F_{fam})$ was obtained from standard tables of the $F$ distribution.

Assuming that the families tested consist of half-sibs the narrow sense individual heritability in retrospect was computed as (additive genetic variance $= 4 \times \sigma^2_{fam}$):

$$\hat{h}^2 = \frac{4 \times \hat{\sigma}^2_{fam}}{4 \times \hat{\sigma}^2_{fam} + \hat{\sigma}^2_{plot} + \hat{\sigma}^2_w} \quad (3)$$

and compared to the known true value ("hats" are used for estimates of theoretical values). The heritability calculations reflect the fact that all the within-plot variance is assumed to be non-genetic. Family mean repeatability was computed as:

$$\hat{h}^2_{fam} = \frac{\hat{\sigma}^2_{fam}}{\hat{\sigma}^2_{fam} + \hat{\sigma}^2_{plot}/n_{rep} + \hat{\sigma}^2_w/(n_{size} \times n_{rep})}. \quad (4)$$

Plot sizes in the experimental designs included the common 1 × 1, 2 × 2, 3 × 3, 5 × 1 and 10 × 1 configurations, while the number of replications were 8, 20, 32 and 44 for the single unit (1 × 1) plot design and 2, 3, 4 and 5 in the multi-unit plot designs. The number of families in the trials came to 30, 50, 70 and 90.

**Table 1.** Form of ANOVA for the model in Eq. (2). $\varrho_w$ = intra-plot correlation coefficient

| Source of variation | *df* | MS | EMS |
|---|---|---|---|
| Replications | $(n_{rep}-1)$ | $MS_r$ | $\sigma_w^2 + n_{size}\,\sigma_{plot}^2 + n_{size}\,n_{fam}\,\sigma_{rep}^2$ |
| Families | $(n_{fam}-1)$ | $MS_f$ | $\sigma_w^2 + n_{size}\,\sigma_{plot}^2 + n_{size}\,n_{rep}\,\sigma_{fam}^2$ |
| Families × replications (plot) | $(n_{fam}-1)\times(n_{rep}-1)$ | $MS_p$ | $\sigma_w^2 + n_{size}\,\sigma_{plot}^2 = \sigma^2(1+(n_{size}-1)\,\varrho_w)$ |
| Within-plots | $(n_{size}-1)\times n_{rep}\times n_{fam}$ | $MS_w$ | $\sigma_w^2 = \sigma^2(1-\varrho_w)$ |

Family values ($\tau$) that would generate a heritability of 0.15 in a trial of half-sibs (i.e., $\hat{\sigma}_f^2 = 0.044$) were generated for all experimental designs (deemed realistic for trials with forest trees (Zobel and Talbert 1984)). Additional levels of heritability (0.25, 0.35 and 0.55) were included in the analysis of the commonly used $5\times1$ plot design. Each of the above 128 design combinations [4(replications) · 4(families) · (5(plot sizes) · 1(heritability levels) +1(plot size) · 3(heritability levels)) = 128] was simulated 1,000 times for each of six levels of $\varrho$ ($\varrho = 0.0, 0.1, \ldots, 0.5$) for a total of 768,000 simulations.

ANOVA residuals ($\varepsilon$) estimated via the model in Eq. (2) can be used to obtain approximate (*posterior*) estimates of the microsite effect for the experimental unit at location $(x, y)$. A weighted sum of the residuals estimated for the eight nearest neighbors (NN) to location $(x, y)$ is frequently used to obtain this estimate of microsite effects (Binns 1987; Correll and Anderson 1983; Kempton and Howes 1981; Loo-Dinkins et al. 1990; Magnussen 1990; Wilkinson et al. 1983). Differences among applied procedures rest with the choice of weights given to the NN-residuals. By subtracting this weighted NN-residual sum from the original observations ($U_{ijk}$) a microsite-adjusted observation is estimated that can undergo a repeated ANOVA according to model (2). It is hypothesized that this NN-adjustment will effectively correct for the bias in effect estimates due to the spatial correlation process. Let $\varepsilon_{NN}(i,j,k)$ denote the weighted sum of the nearest neighbor residuals estimated for an observation $U_{ijk}$, and let $U_{ijk\,|\,NN}$ denote the NN-adjustment observation; we then have:

$$U_{ijk\,|\,NN} = U_{ijk} - \varepsilon_{NN}(i,j,k) = \mu + \tau_i + \beta_j + \pi_{ij} + \varepsilon_{ijk} - \varepsilon_{NN}(i,j,k)\,. \tag{5}$$

Seven different weight factors ($b$) were tried in the calculations of the weighted sum of NN-residuals. Essentially, $b$ is the slope of the regression between the residual of an experimental unit and those of its neighbors. All experimental units were assigned eight nearest neighbors. Missing neighbors along outside edges were replaced by their interior complement. The first chose (B1) had $b = \varrho_N/8$ where $\varrho_N$ is the average correlation of residuals (determined from the first ANOVA pass) among eight NN experimental units. The second choice (B2) had $b = \varrho_{ols}/8$ where $\varrho_{ols}$ is the average first-order correlation of residuals as estimated from the ANOVA residuals (an ordinary least-squares estimate of $\varrho$). The third choice (B3) was based on an iterated adjustment procedure starting with $b = \varrho_{ols}/8$. It is clear from Eq. (5) that the NN-adjusted phenotypic values ($U_{ijk\,|\,NN}$) are, in effect, obtained from a change in the residuals, Equation (5) describes a simultaneous autoregressive scheme of the residuals. The microsite-adjusted observations obtained via the first ANOVA pass are therefore only approximate. An iterative process of estimation and adjustment of the true but unknown residuals would give the desired final adjustment (Besag 1974). With an initial choice of $b < 1$ the final iterated weight (slope) becomes $b/(1-b)$ as shown by Magnussen and Yeatman (1988). With $b = \varrho_{ols}/8$ the final weight in the third option (B3) for $b$ equalled $\varrho_{ols}/(8\cdot(1-\varrho_{ols}))$. The fourth choice (B4) of $b$ was similar to B3 only with $\varrho_{ols}$ replaced by $\varrho$, the true spatial AR1 correlation. In practice only $\varrho_{ols}$ will be available, but as shall be shown later this estimate of the spatial correlation is always biased towards randomness (Cochrane and Orcutt 1949). The fifth value of $b$ (B5) was derived from a transformation of the AR1 process of environmental values ($U_{x,y}$) to a simultaneous regression scheme of the form:

$$U_{(x,y)} = U_{(x,y)\,|\,NN} + \lambda \times \sum_{(x',y')\in NN(x,y)} U_{(x',y')}\,, \tag{6}$$

where $NN(x, y)$ denotes the four immediate row and column neighbors (the four diagonal neighbors have been omitted) to the unit at location $(x, y)$. Lambda ($\lambda$) in Eq. (6) is also called Mead's coefficient of inter-plant interaction (Mead 1967). Magnussen gives an algorithm to estimate $\lambda$ from the intra-plot correlation $\varrho_w$ (Magnussen 1989a, eq. (11) on p. 373. See Table 1 for estimation procedures of $\varrho_w$). With $\lambda$ obtained this way, the fifth choice (B5) for $b$ became $b = 0.5\lambda + 0.5\lambda^2$; the last term was added to account for the four diagonal neighbors. The sixth choice (B6) for $b$ was also based on a transformation of the AR1 process to a simultaneous regression scheme, only this time Bartlett's transformation rule $b = 2\varrho/(8\cdot(1+\varrho^2))$ was used instead (Bartlett 1978). The final choice of $b$ (B7) consisted of $b = 1/8$, in other words the NN-adjustment is based on the simple average of the eight nearest neighbor residuals. This method is known as either Papadakis method (see Papadakis (1984) for reference to the original method from 1937) or the moving average method (for example Wright 1978).

A positive spatial AR1 autocorrelation ($\varrho$) creates a positive intra-plot correlation ($\varrho_w$) in the multi-unit plot designs, which leads to the relationships between the among- and within-plot variances outlined in Table 1 (see also Magnussen 1989a; Snedecor and Cochran 1971). The theoretical relationship between $\varrho_w$ and $\varrho$ takes the following form for the investigated multi-unit plots:

$$2\times2 \text{ plots:} \quad \varrho_w = (2\times\varrho + \varrho^2)/3\,, \tag{7}$$

$$3\times3 \text{ plots:} \quad \varrho_w = (24\times\varrho + 28\times\varrho^2 + 16\times\varrho^3 + 4\times\varrho^4)/72, \tag{8}$$

$$n\times1 \text{ plots:} \quad \varrho_w = \left(\sum_{i=0}^{n-1}(n-i)\times\varrho^i\right)\Big/\left(\sum_{i=0}^{n-1}(n-i)\right). \tag{9}$$

Observed intra-plot correlations ($\hat{\varrho}_w$) were, on the average, 1% below the theoretical values derived from the nominal values of $\varrho$ and Eqs. (7)–(9) ($\mathrm{corr}(E(\varrho_w), \hat{\varrho}_w) = 0.998$).

Simulated phenotypic correlations among first-order neighbor values ($U_{x,y}$) within the same row or column were, on an average, across all designs (i.e., 768,000 simulated trials) 2–5% below their nominal value of $\varrho$. This discrepancy was attributed to the lack of a correction for finite population sizes in the calculations of variances and covariances, the downward bias towards randomness in *a posteriori* least-squares estimates of autocorrelations (Cochrane and Orcutt 1949) and the complex pattern of "genetic" carry-over effects from one location to the next. Relationships between some of the various spatial measures of NN-correlations in the designs with a $5\times1$ plot design have been listed in Table 2.

**Table 2.** Estimates of spatial relationships in the 5 × 1 plot design

| $\varrho$ | $\varrho_{ols}$ [a] | $\varrho$ [b] | $\varrho_N$ [c] | $\lambda$ [d] |
|---|---|---|---|---|
| 0.1 | 0.06 | 0.04 | 0.08 | 0.04 |
| 0.2 | 0.15 | 0.10 | 0.13 | 0.08 |
| 0.3 | 0.25 | 0.16 | 0.20 | 0.12 |
| 0.4 | 0.34 | 0.25 | 0.27 | 0.16 |
| 0.5 | 0.44 | 0.35 | 0.36 | 0.19 |

[a] First-order autocorrelation determined from ANOVA residuals
[b] Average correlation among first-order neighbors (estimated directly from the simulated values)
[c] Average correlation among the eight NN neighbors of a single unit
[d] Mead's coefficient of inter-plant interaction

**Table 3.** Estimated regression coefficients and fit statistics for the model $\sigma^2_{fam} = s_0 \cdot \exp(a \cdot \varrho + b \cdot n_{rep} + c \cdot n_{fam})$. Standard errors of estimates are indicated in parentheses

| Design | $h^2$ [a] | $s_0 \cdot 10^2$ | $a$ | $b \cdot 10^3$ | $c \cdot 10^4$ | RSE [b] $\cdot 10^3$ | $R^2$ [c] |
|---|---|---|---|---|---|---|---|
| 2 × 2 | | | 1.71 (0.02) | 8.1 (2.0) | 5.5 (1.1) | | |
| 3 × 3 | | | 2.77 (0.01) | 1.5 (1.5) | 4.1 (0.8) | | |
| | 0.15 | 4.2 (0.03) | | | | 2.1 | 0.996 |
| 5 × 1 | | | 1.88 (0.02) | −4.9 (2.0) | 4.6 (1.1) | | |
| 10 × 1 | | | 2.33 (0.02) | −7.9 (1.8) | 2.6 (1.0) | | |
| | 0.15 | 4.2 (0.04) | | | | | |
| | 0.25 | 7.9 (0.01) | | | | | |
| 5 × 1 | | | 1.88 (0.01) | −0.8 (1.6) | 4.2 (0.8) | 5.0 | 0.995 |
| | 0.35 | 12.8 (0.02) | | | | | |
| | 0.55 | 28.9 (0.05) | | | | | |

[a] Narrow sense individual heritability (known)
[b] Root mean square error of regression
[c] Fraction of the total variance explained by the fitted model

## Results

Estimates of family variance components and thus estimates of additive genetic variance were upward biased in multi-unit plot designs with a positive spatial autocorrelation among neighboring units. The average bias in experiments with a true heritability of 0.15 (true $\sigma^2_f = 0.044$) is displayed in Fig. 2. An exponential rise in the bias with an increasing spatial autocorrelation ($\varrho$) is evident. Each of the curves in Fig. 2 represents an average of 16,000 simulations with various levels of replications and number of families (see the Methods section for details). Square 3 × 3 row plots and 10 × 1 row plots produced the largest bias for $\varrho > 0$, while the 2 × 2 and the 5 × 1 plot designs displayed a lower inflation rate. Designs with single-unit plot designs were, on the average, free from any systematic bias in the estimated family variance component. A ranking of the bias visualized in Fig. 2 would closely follow the ranking of the inflation factor for plots ($= n_{size}/(1-\varrho_w)$; see also Table 1 for details).

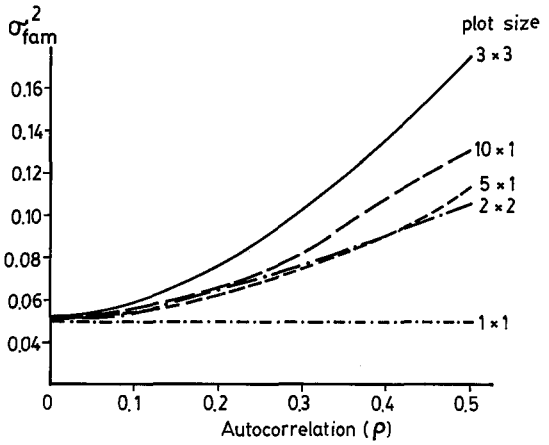


**Fig. 2.** Trends in family variances at various levels of autocorrelation. True $\sigma^2_f = 0.044$

A model-based quantification of the bias in family variance components due to spatial autocorrelation among neighboring units is presented in Table 3. The highly significant ($P < 0.001$) regression coefficient $a$ encapsulates the relative inflation in the family variance component due to $\varrho$, while the coefficients $b$ and $c$ measure the influence of replications and the number of families, respectively. For every 0.1 increase of $\varrho$ in the multi-unit plot designs the family variance estimate goes up by 17–28%. Realistic values of $\varrho$ in field trials can be expected to fall in the 0.0–0.3 range (Magnussen 1989a, 1990). Hence, the potential inflation of the family variance may be as high as 84%. All four multi-plot designs gave rise to statistically distinct rates of inflation ($P < 0.001$). Replications had a surprisingly weak influence on the bias caused by $\varrho$. A rather modest, albeit statistically significant, 0.5–0.8% drop in the bias could be achieved for every added replication in the designs with five-tree or ten-tree row plots. Bias in trials with square plot designs could not be reduced by adding more replicates, and the results indicate that the bias may actually increase slightly (0.2–0.8%) with added replications. No two designs had the same effect of replications ($P < 0.05$). Changing the number of families tested had little effect on the family variance, but adding say 50 families to a multi-unit plot design would inflate the family variance component by 1–3%.

Inflation rates of the family variance component were independent of the heritability level in the experimental population (Table 3). Separate regressions for each of the

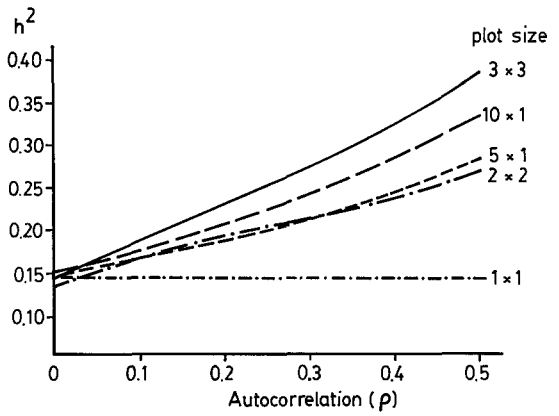


**Fig. 3.** Trends in heritability estimates with increasing autocorrelation. True $h^2 = 0.15$

heritability levels 0.15, 0.25, 0.35 and 0.55 in the $5 \times 1$ designs gave results that could safely be combined without a significant loss of information (likelihood ratio test: $F_{obs}(12,380) = 1.03$, $P(F > F_{obs}) > 0.25$).

An inflation of the family variance component translates into an inflation of the estimated heritability (Fig. 3). Even a modest autocorrelation of 0.2 caused a positive bias of 30–36% in the estimated heritability when the true heritability was 0.15. Purely numerical considerations would show that the relative bias of heritabilities due to $\varrho$ would be less severe at higher levels of additive variance, despite the fact that the relative inflation of the family variance would remain the same. Estimates of breeding values will be upward biased by an amount equal to the square root of the inflation factors of the family variances or roughly one-half of the *a*-values in Table 3.

Family mean repeatabilities (Table 4) and the *F*-ratios constructed to test $\hat{\sigma}^2_{fam} = 0$ were, by and large, unaffected by $\varrho$ (changes less than 5%). This illustrates that autocorrelation modifies the plot mean-square and the family mean-square in equal proportions.

Reductions of positive bias in the estimated family variance component due to $\varrho$ could be achieved by redoing the ANOVA, after having subtracted from the original observations ($U_{ijk}$) the weighted residuals (from the first ANOVA pass) of neighboring units multiplied by a regression slope *b* (see also Methods section). Results obtained with seven different values of *b* are listed in Table 5 along with the results of doing no neighborhood adjustment (B0). Best overall results in terms of minimum bias arose from letting the covariance slope factor *b* be equal to the weighted average ($\lambda_N = 0.5\lambda + 0.5\lambda^2$, see under B5 in Table 5) of Mead's coefficient ($\lambda$) of inter-plant interaction (Mead 1967). Family variance components derived from this procedure were only 9–11% too high, a marked drop from the inflation of 17–149% encountered in the absence of any adjustment (see under B1 in Table 5). Bartlett's (1978) translation of the autoregressive model (AR1) of residuals into a symmetric nearest neighbor regression model (see under B6 in Table 5) should be preferred as an adjustment slope when $0 < \varrho \leq 0.2$ because it then yields a slightly (~2%) superior reduction in the bias of the family variance. Although the use of simple estimates of $\varrho$ (either $\varrho_{ols}$ or the average correlation among first-order neighbors $\varrho_N$, see under B2 and B3 in Table 5) gave impressive reductions in the bias, they were, nevertheless, quite inferior to the aforementioned alternatives. An iteration of the adjustment procedure (using $b = \varrho$ or $b = \varrho_N$, see under B4 and B5 in Table 5) proved to be more efficient than the non-iterated adjustment. Adjustments based on the simple residual average of neighboring [Papadakis' method, see Papadakis (1984) for details] units induced a strong negative bias (6–32%, see under B7 in Table 5) in the estimated family variance components. The full adjustment ($b = 1/8$) is only acceptable at high $\varrho$ values (>0.4).

**Table 4.** Effect of spatial autocorrelation on family mean repeatability

| Plot size | $h^2_{fam}$ (SE) | |
|---|---|---|
| | $\varrho = 0$ | $\varrho = 0.05$ |
| 1 × 1 | 0.47 (0.04) | 0.47 (0.04) |
| 2 × 2 | 0.35 (0.02) | 0.35 (0.02) |
| 3 × 3 | 0.55 (0.02) | 0.57 (0.02) |
| 5 × 1 | 0.40 (0.02) | 0.42 (0.02) |
| 10 × 1 | 0.57 (0.02) | 0.59 (0.02) |

**Table 5.** Family variances after adjustments of spatial autocorrelation with various slopes (B0–B7) in the analysis of covariance. Table entries are family variances in percent of unadjusted family variance at $\varrho = 0$ (reference level 100). Plot size = $5 \times 1$

| $h^2$ | $\varrho$ | B0 [a] | B1 [b] | B2 [c] | B3 [d] | B4 [e] | B5 [f] | B6 [g] | B7 [h] |
|---|---|---|---|---|---|---|---|---|---|
| 0.35 | 0.1 | 117 | 113 | 114 | 112 | 110 | 107 | 105 | 68 |
| | 0.2 | 139 | 129 | 130 | 128 | 119 | 111 | 109 | 73 |
| | 0.3 | 168 | 146 | 143 | 138 | 122 | 111 | 111 | 79 |
| | 0.4 | 203 | 166 | 160 | 144 | 115 | 110 | 113 | 86 |
| | 0.5 | 249 | 184 | 174 | 138 | 95 | 105 | 115 | 94 |
| 0.55 | 0.1 | 118 | 112 | 114 | 114 | 110 | 107 | 105 | 68 |
| | 0.2 | 140 | 127 | 129 | 127 | 118 | 110 | 109 | 73 |
| | 0.3 | 168 | 144 | 143 | 137 | 121 | 111 | 111 | 79 |
| | 0.4 | 204 | 161 | 158 | 143 | 115 | 109 | 112 | 86 |
| | 0.5 | 249 | 177 | 172 | 137 | 94 | 104 | 114 | 94 |

[a] $b = 0$ (no adjustment)
[b] $b = \varrho_N/8$ (average neighborhood correlation)
[c] $b = \varrho_{ols}/8$ (residual correlation among first-order neighbors as determined from ANOVA)
[d] $b = \varrho_{ols}/[8 \cdot (1 - \varrho_{ols})]$
[e] $b = \varrho/[8 \cdot (1 - \varrho)]$
[f] $b = \lambda_N$ ($\lambda_N = (4\lambda + 4\lambda^2)/8$)
[g] $b = 2\varrho/[8 \cdot (1 + \varrho^2)]$
[h] $b = 1/8$

Reduction of bias was achieved without a great increase in the standard error of the adjusted estimates of family variance and heritability. NN-adjusted estimates had a relatively higher standard deviation than did unadjusted estimates. Averaged over all the simulations the coefficient of variation of family variance components increased from 24.9% to 29.2% due to the NN-adjustments. The corresponding figures for the narrow-sense individual heritability were 23.3% and 31.4%, respectively.

## Discussion

Positive correlations among neighboring experimental units sharing common microsites must be expected as the norm in genetics field trials, rather than as the exception (Besag and Kempton 1986; Cliff and Ord 1981; Upton and Fingleton 1985). An exact causal formulation of this correlation process is not possible in genetic trials where observations at a single location is the outcome of complex genotype × environment interactions. In genetic trials the estimated genetic values are conditional on the environments (i.e., microsites) that the genotype has been exposed to and the reference population used in the test. A clear-cut separation into "genetic" and "environmental" effects in trials with phenotypic correlation among neighboring units is, therefore, not possible. This problem is attenuated in trials with genetically heterogeneous material. The adopted simulation process confounded – in a complex way – the true genetic effects with the environmental effects in an attempt to create a "realistic" scenario that models "closely" the real world. A first-order autoregressive process model was chosen because it reflects reality quite well when microsite differences are caused by: (1) dispersal from a few sources; (2) large favorable patches; or (3) a gradient (Cliff and Ord 1981). Empirical evidence support this type of "spatial process" (for example, Binns 1987; Cullis and Gleeson 1989; Besag and Kempton 1986; Magnussen 1990; Modjeska and Rawlings 1983; Smith 1938). Single-unit plot designs will, under these confounded circumstances, be the only type of design that will provide the analyst with uninflated estimates of genetic variances and gain (Libby and Cockerham 1980; Loo-Dinkins et al. 1990). However, single-unit plot designs quickly become the worst in terms of inflating genetic variances and heritabilities (Magnussen 1989 b; Stern 1968) when neighboring units compete for resources, notably light. Different designs are therefore needed for short-term and long-term trials (Edwards 1956).

Simulation of field experiments appears to be the only tractable approach to quantify the influence of spatial autocorrelation on estimated family variance components (Stroup and Mulitze 1991; Wilkinson et al. 1983). The underlying AR1 scheme chosen to generate phenotypic correlations is very simple (no trend, same correlation along two axes, only the immediate NN effects are needed in the model, no differential effects of neighbors and spatial effects only expressed at the phenotypic level). Realized correlation patterns in field trials may indeed resemble those of an AR1 process (Magnussen 1990) even if no well-defined process exists *per se*. NN-correlations will typically fluctuate between areas within an experiment, and correlations will often be stronger in one direction than in another (Correll and Cellier 1987; Modjeska and Rawlings 1983). Regardless of the actual process that generates the positive spatial autocorrelation among neighboring units, the results will be an inflation of the genetic variance components in a way similar to the results presented in this study.

The genetic model entertained in the simulated trials contained only additive effects at the family level. Adding within-family genetic effects would make no overall difference inasmuch as these effects remain non-estimable. Simulations done with a random within-family component gave virtually identical inflation rates for the family variance components and the heritability estimates. Non-additive genetic effects were not considered, but simple considerations of their estimation (Falconer 1981; Gallais 1976; Hallauer and Miranda 1981; Wright 1982) suggest that they would be even more seriously biased by spatial autocorrelations than the additive effects.

Family variance components obtained from multi-unit plot designs will, in most cases, need a downward adjustment before realistic estimates of the amount of additive genetic variance can be made. Most field trials can be expected to show an autocorrelation of 0.05–0.30 between first-order neighbors (as estimated from uniformity trials, Magnussen 1989 a), and the inflation of the initial estimate of the family variance could be as high as 84%. Additional replications will not be an effective way to solve this problem. The simple Papadakis adjustment procedure proved to be quite effective given the right choice of slope in the covariance regression. Although the NN-adjustment leads to an increase (5–8%) in the relative standard error of the genetic estimates, the reduction in bias outweighs this drawback.

Papadakis' method from 1937 (see Papadakis 1984 for details) is not only intuitively appealing but also easy to implement. Care must be given to the choice of slope in the NN-covariance adjustment. A full NN-adjustment (slope equal to one) as pioneered by Papadakis and Wright (1978) is only effective in the presence of a strong spatial autocorrelation ($\varrho > 0.45$); at common low levels of $\varrho$ ($\varrho < 0.15$) it may actually be worse than no adjustment. Earlier criticism of a lack of numerical stability raised against the iterated Papadakis method (Binns 1987; Correll and Anderson 1983; Wilkinson et al. 1983) is mitigated by the results in this study. The fact that the final iterated slope was obtained analytically and not through

use of actual estimates explains this positive experience with the iterated procedure. There are several adjustment procedures in which the autoregressive scheme has been transformed into a simultaneous regressive scheme (Bartlett 1978; Besag 1974; Ord 1975). Bartlett (1978) and Magnussen (1989 a) have shown how to obtain the coefficients of the simultaneous regressive schemes with a minimum of added effort. Other NN-adjustment methods may, of course, prove to be equally efficient, but a full scale comparison is beyond the scope of this study. Several maximum likelihood methods, however, would require excessive computer resources for designs of practical relevance unless plot means were used as the basic data for the analyses. NN-adjustments at the plot level are likely to be less efficient than NN-adjustment of single observations because a higher proportion of plots will have a reduced set of nearest neighbors. NN-adjustment at the individual level should also be more efficient in adjusting for highly irregular mosaics of microsites that do not coincide with the actual layout of plots.

This study will hopefully help remove some of the lingering scepticism against NN-adjustment procedures in applied analysis of field experiments. Reliance on inflated estimates of genetic variance components creates unrealistic expectations with the prospect of inefficient investments of scarce resources.

*Acknowledgements.* Helpful comments and critique of an earlier version of this paper were kindly provided by Drs. H. P. van Buijtenen, R. L. Correll, P. Clarke, and two anonymous journal referees.

## References

Bartlett MS (1978) Nearest neighbor models in the analysis of field experiments. J R Stat Soc B 40:147–174

Besag J (1974) Spatial interaction and the statistical analysis of lattice systems. J R Stat Soc B 36:192–236

Besag J, Kempton R (1986) Statistical analysis of field experiments using neighboring plots. Biometrics 42:231–251

Binns MR (1987) Practical use of neighbor methods and alternatives in the analysis of field trials. Can J Plant Sci 67:477–489

Brockwell JP, Davis RA (1987) Time series: theory and methods. Springer, Berlin Heidelberg New York

Cliff AD, Ord JK (1981) Spatial processes. Pion, London

Cochrane D, Orcutt GH (1949) Application of least squares regression to relationships containing autocorrelated error terms. J Am Stat Assoc 44:32–61

Correll RL, Anderson RB (1983) Removal of intervarietal competition effects in forestry varietal trials. Silvae Genet 32:162–165

Correll RL, Cellier KM (1987) Effects of plot size block size and buffer rows on the precision of forestry trials. Aust For Res 17:11–18

Cullis BR, Gleeson AC (1989) Efficiency of neighbor analysis for replicated variety trials in Australia. J Agric Sci 113:233–239

Edwards MV (1956) The design layout and control of provenance experiments. Silvae Genet 5:169–180

Falconer DS (1981) Introduction to quantitative genetics, 2nd edn. Longman, New York

Gallais A (1976) Effects of competition on means, variances, and covariances in quantitative genetics with application to general combining ability selection. Theor Appl Genet 47:189–195

Gregorius H-R, Namkong G (1987) Resolving the dilemmas of interaction, separability, and additivity. Math Biosci 85:51–69

Hallauer AR, Miranda JB (1981) Quantitative genetics in maize breeding. Iowa State University Press, Ames, Iowa

Kempton RA, Howes (1981) The use of neighboring plot values in the analysis of variety trials. Appl Stat 30:59–70

Libby WJ, Cockerham CC (1980) Random non-contiguous plots in interlocking field layouts. Silvae Genet 29:183–190

Loo-Dinkins JA, Tauer CG, Lambeth CC (1990) Selection system efficiencies for computer simulated progeny test field designs in loblolly pine. Theor Appl Genet 79:89–96

Magnussen S (1989 a) Inter-plant interactions and their influence on within- and among-plot variances. Scand J For Res 4:369–377

Magnussen S (1989 b) Effects and adjustments of competition bias in progeny trials with single-tree plots. For Sci 35:537–547

Magnussen S (1990) Application and comparison of spatial models in analyzing tree genetics field trials. Can J For Res 20:536–546

Magnussen S, Yeatman CW (1988) Adjusting for inter-row competition in a jack pine provenance trials? Silvae Genet 36:206–214

Mead R (1967) A mathematical model for the estimation of inter-plant competition. Biometrics 23:189–205

Modjeska JS, Rawlings JO (1983) Spatial correlation analysis of uniformity trials. Biometrics 39:373–384

Monserud RA, Rehfeldt GE (1990) Genetic and environmental components of site index in inland Douglas-fir. For Sci 36:1–9

Ord K (1975) Estimation methods for models of spatial interaction. J Am Stat Soc 70:120–126

Papadakis JS (1984) Advances in the analysis of field experiments. Proc Acad Athens 59:326–342

Smith HF (1938) An empirical law describing heterogeneity in the yields of agricultural crops. J Agric Sci 28:1–23

Snedecor GW, Cochran WG (1971) Statistical methods, 6th edn. Iowa University Press, Ames, Iowa

Stein A, Corsten LCA (1991) Universal kriging and cokriging as a regression procedure. Biometrics 47:575–587

Stern K (1965) Vollständige Varianzen und Kovarianzen in Pflanzenbeständen. Silvae Genet 14:6–11

Stern K (1968) Überlegungen zur optimalen Teilstückgrössen in Feldversuchen mit Waldbäumen. Silvae Fenn 2:248–260

Stroup WW, Mulitze DK (1991) Nearest neighbor adjusted best linear unbiased prediction. Am Stat 45:194–200

Upton GJG, Fingleton B (1985) Spatial analysis by example. John Wiley, New York

Wilkinson GN, Eckert SR, Hancock TW, Mayo O (1983) Nearest neighbor (NN) analysis of field experiments. J R Stat Soc B 45:151–211

Wright AJ (1982) Some implication of a first-order model of inter-plant competition for the means and variances of complex mixtures. Theor Appl Genet 64:91–96

Wright JW (1978) An analysis method to improve statistical efficiency of a randomized complete block design. Silvae Genet 27:12–14

Zimmerman DL, Harville DA (1991) A random field approach to the analysis of field-plot experiments and other spatial experiments. Biometrics 47:223–239

Zobel BJ, Talbert JT (1984) Applied forest tree improvement. John Wiley, New York