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Sampling distributions, biases, variances, and confidence intervals for genetic correlations

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Abstract Genetic correlations (ρ_g) are frequently estimated from natural and experimental populations, yet many of the statistical properties of estimators of ρ_g are not known, and accurate methods have not been described for estimating the precision of estimates of ρ_g. Our objective was to assess the statistical properties of multivariate analysis of variance (MANOVA), restricted maximum likelihood (REML), and maximum likelihood (ML) estimators of ρ_g by simulating bivariate normal samples for the one-way balanced linear model. We estimated probabilities of non-positive definite MANOVA estimates of genetic variance-covariance matrices and biases and variances of MANOVA, REML, and ML estimators of ρ_g, and assessed the accuracy of parametric, jackknife, and bootstrap variance and confidence interval estimators for ρ_g. MANOVA estimates of ρ_g were normally distributed. REML and ML estimates were normally distributed for $\rho_g = 0.1$, but skewed for $\rho_g = 0.5$ and 0.9. All of the estimators were biased. The MANOVA estimator was less biased than REML and ML estimators when heritability (H), the number of genotypes (n), and the number of replications (r) were low. The biases were otherwise nearly equal for different estimators and could not be reduced by jackknifing or bootstrapping. The variance of the MANOVA estimator was greater than the variance of the REML or ML estimator for most H, n, and r. Bootstrapping produced estimates of the variance of ρ_g close to the known variance, especially for REML and ML. The observed coverages of the REML and ML bootstrap interval estimators were consistently close to stated coverages, whereas the observed coverage of the MANOVA bootstrap interval estimator was unsatisfactory for some H, ρ_g, n, and r. The other interval estimators produced unsatisfactory coverages. REML and ML bootstrap interval estimates were narrower than MANOVA bootstrap interval estimates for most H, ρ_g, n, and r.

Key words Bootstrap · Jackknife · Heritability · Selection indices

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Introduction

The biological or economic merit of an individual, family, or line is usually a function of two or more traits. The genetic correlations (ρ_g) between traits can be used to assess the consequences of selecting for one or more traits. Optimum multiple-trait phenotypes can be produced for both uncorrelated traits and correlated traits when the genetic correlation is caused by genes linked in repulsion and the distances between the genes are sufficient to produce recombinant genotypes. A genetic correlation is antagonistic (1) when the correlation is predominantly caused by pleiotropic genes where the superior alleles for one trait are the inferior alleles for the other trait (antagonistic pleiotropy) or (2) when the correlation is predominantly caused by non-pleiotropic genes linked in repulsion. When a genetic correlation is strongly antagonistic and entirely caused by pleiotropy, the phenotypes cannot be optimum for both traits and the breeder is faced with two choices, either employing compromising selection for each trait or optimizing selection for one trait over the other (Baker 1986). This compromise is not necessary for strongly antagonistic genetic correlations entirely caused by repulsion linkages between loci affecting different traits.

Breeders use genetic correlations to (1) estimate indirect and correlated selection responses, (2) estimate selection indices, and (3) assess the consequences of selecting correlated traits (Falconer 1981; Lande 1984; Baker 1986). Despite the importance of multiple-trait

selection and genetic correlations to most breeding programs, many of the statistical properties of genetic correlation estimators and the precision of estimates of genetic correlations are not known.

Several estimators of the sampling errors of ρ_g have been described (Reeve 1955; Robertson 1959; Tallis 1959; Scheinberg 1966; Falconer 1981), but they are seldom used, and their statistical accuracy has not been assessed. Riska et al. (1989) used bias-corrected percentile bootstrap confidence intervals to estimate the precision of multivariate analysis of variance (MANOVA) estimates of genetic correlations. Other methods for estimating confidence intervals for genetic correlations have not been used, and the statistical accuracy of confidence interval estimation methods has not been assessed.

MANOVA has been widely used for estimating variance-covariance matrices and other parameters of multivariate linear models (Searle 1970). Historically, MANOVA has been more widely used by plant breeders than restricted maximum likelihood (REML) (Amemiya 1985) and maximum likelihood (ML) methods for univariate problems. REML and ML have not been used for multivariate problems because of computational difficulties and the lack of software. Although the statistical properties of ANOVA, REML, and ML estimators of variances have been carefully studied (Swallow and Monahan 1984), the statistical properties of genetic correlations have not been described.

The variance of the ML estimator of the between-class variance is typically smaller than the variances of the ANOVA and REML estimators of the between-class variance for the one-factor linear model (Swallow and Monahan 1984). Correcting for bias, however, increases the variance and eliminates the superiority of the ML estimator (Swallow and Monahan 1984). Swallow and Monahan (1984) proposed the use of ANOVA estimators when $\sigma_b^2/\sigma_\omega^2 > 0.5$, where σ_b^2 and σ_ω^2 are the between- and within-class variances for the one-factor linear model, because ANOVA solutions are linear, and the probability of negative ANOVA estimates of the between-class variance (Searle 1970) decreases as σ_g^2/σ_e^2 increases.

MANOVA, however, produces non-positive definite (NPD) estimates of the genetic (between-class) variance-covariance matrix (Σ_g) (Hill and Thompson 1978). This is a major drawback of the method. The probability of NPD estimates of Σ_g is a function of negative variance estimates and of out-of-bounds estimates ($-1.0 \leqslant \rho_g \leqslant 1.0$). MANOVA can yield meaningless estimates ($\hat{\rho}_g < -1$ or $\hat{\rho}_g > 1$), or no estimate, when the genetic variance for one of the traits is negative, which happens frequently for low-heritability traits (Searle 1970; Knapp and Bridges 1988). The probability of NPD estimates range from 0.0 to 1.0 for $\rho_g = 0$ (Hill and Thompson 1978). The probability of NPD estimates increased as the number of dependent variables (traits) increased and the heritabilities of the traits decreased. As with univariate linear models, this problem is eliminated by using REML or ML estimators because these methods produce solutions in the parameter space $0.0 < \sigma^2 <$ infinity (Harville 1977; Rao and Kleffe 1988).

Parametric confidence interval estimators are often difficult to define for parameters with complicated or unknown distributions (Knapp and Bridges 1988; Knapp et al. 1989; Bridges et al. 1990; Mitchell-Olds and Bergelson 1990). Jackknifing and bootstrapping are routinely used (1) for parameters with unknown or undefined distributions, (2) when parametric methods produce inaccurate estimates, or (3) when a robust estimator has merit (Miller 1974; Efron 1982). Jackknifing and bootstrapping have been used to estimate the precision of estimates for a wide range of genetic parameters (Weir 1990), among them the between-class variance (Miller 1974), outcrossing rates (Schoen and Clegg 1986; Knapp et al. 1991), heritabilities and selection gains (Arvesen and Schmitz 1970; Knapp et al. 1989; Bridges et al. 1990), and genetic correlations (Riska et al. 1989).

Our objectives were to (1) describe some of the properties of multivariate analysis of variance (MANOVA), restricted maximum likelihood (REML), and maximum likelihood (ML) estimators of ρ_g and (2) assess the accuracy of parametric, jackknife, and bootstrap variance and confidence interval estimators for genetic correlations.

Materials and methods

Model, parameters, and estimators

We simulated bivariate normal observations for the balanced one-factor linear model. The model for t traits is

$$y_{ijk} = \mu_i + g_{ij} + e_{ijk} \quad (1)$$

where the y_{ijk} are observations of the kth replication of the jth genotype for the ith trait, $i = 1, 2, \ldots, t, j = 1, 2, \ldots, n, k = 1, 2, \ldots, r, n$ is the number of genotypes, r is the number of replications, μ_i is the population mean for the ith trait, g_{ij} is the random effect of the jth genotype for the ith trait, and e_{ijk} is the random effect of the kth replication of the jth genotype for the ith trait. g_{1j} and g_{2j} are correlated random variables with zero means and a variance-covariance matrix

$$\Sigma_g = \begin{bmatrix} \sigma_{g_1}^2 & \sigma_{g_{12}} \\ \sigma_{g_{12}} & \sigma_{g_2}^2 \end{bmatrix},$$

where $\sigma_{g_1}^2$ is the genetic variance for the first trait, $\sigma_{g_2}^2$ is the genetic variance for the second trait, and $\sigma_{g_{12}}^2$ is the covariance between the first and second traits. e_{1jk} and e_{2jk} are correlated random variables with zero means and a variance-covariance matrix

$$\Sigma_e = \begin{bmatrix} \sigma_{e_1}^2 & \sigma_{e_{12}} \\ \sigma_{e_{12}} & \sigma_{e_2}^2 \end{bmatrix},$$

where $\sigma_{e_1}^2$ is the error variance for the first trait, $\sigma_{e_2}^2$ is the error variance for the second trait, and $\sigma_{e_{12}}^2$ is the error covariance between the first and second traits. $\rho_g = \sigma_{g_{12}}/(\sigma_{g_1}\sigma_{g_2})$ is the genetic correlation, $\rho_e = \sigma_{e_{12}}/(\sigma_{e_1}\sigma_{e_2})$ is the error correlation, and $H_i = \sigma_{g_i}^2/(\sigma_{g_i}^2 + \sigma_{e_i}^2/r)$ is the family mean heritability for the ith trait.

The mean square and mean product matrix for genotypes is

$$M_g = \begin{bmatrix} M_{g_1} M_{g_{12}} \\ M_{g_{12}} M_{g_2} \end{bmatrix},$$

and for error is

$$M_e = \begin{bmatrix} M_{e_1} M_{e_{12}} \\ M_{e_{12}} M_{e_2} \end{bmatrix},$$

where M_{g_1} is the genotype mean square for the first trait, M_{g_2} is the genotype mean square for the second trait, $M_{g_{12}}$ is the genotype mean product for the first and second traits, M_{e_1} is the error mean square for the first trait, M_{e_2} is the error mean square for the second trait, and $M_{e_{12}}$ is the error mean product for the first and second traits. The expected mean square for genotypes is $E(M_g) = \Sigma_e + r\Sigma_g$, and for error is $E(M_e) = \Sigma_e$.

MANOVA, REML, and ML methods were used to estimate the parameters of model (1) for each simulated sample. The MANOVA estimator of Σ_g is $\hat{\Sigma}_g = [M_g - M_e]/r$ and of Σ_e is $\hat{\Sigma}_e = M_e$; thus, the MANOVA estimtors of the variances and covariances are

$$\hat{\sigma}^2_{e_1} = M_{e_1}, \hat{\sigma}^2_{e_2} = M_{e_2}, \hat{\sigma}_{e_{12}} = M_{e_{12}}, \hat{\sigma}^2_{g_1} = (M_{g_1} - M_{e_1})/r,$$

$$\hat{\sigma}^2_{g_2} = (M_{g_2} - M_{e_2}/r), \quad \text{and} \quad \hat{\sigma}_{12} = (M_{g_{12}} - M_{e_{12}})/r,$$

while the MANOVA estimator of ρ_g is $\hat{\rho}_g = \hat{\sigma}_{g_{12}}/(\hat{\sigma}_{g_1}\, \hat{\sigma}_{g_2})$. If $\hat{\sigma}^2_{g_1} > 0$ and $\hat{\sigma}^2_{g_2} > 0$, then ρ_g is estimable. If $\hat{\sigma}^2_{g_1} \leqslant 0$ or $\hat{\sigma}^2_{g_2} \leqslant 0$, then $\hat{\rho}_g$ is undefined.

ML and REML estimators of genetic variances and covariances are ordinarily estimated using iterative methods; however, Klotz and Putter (1969) and Amemiya (1985) described linear ML and REML solutions for the balanced one-factor linear model. We used these solutions to produce ML and REML estimates.

The ML solution of Σ_g for [1] is

$$\hat{\Sigma}_{g_{\mathrm{ML}}} = (nr)^{-1} M_t\, [M_t^- A]^* \tag{2}$$

where $M_t = (n-1)M_g + n(r-1)M_e$, $A = (n-1)M_g - nM_e$, M_t^- is a generalized inverse of M_t, and $[M_t^- A]^*$ is reduced rank positive semi-definite approximation of $M_t^- A$ (Klotz and Putter 1969). The REML solution of Σ_g for (1) is

$$\hat{\Sigma}_{g\mathrm{REML}} = r^{-1} P_k(\Lambda_k - I)P'_k, \tag{3}$$

where $\Lambda_k = \mathrm{diag}\,[\lambda_1, \lambda_2, \ldots \lambda_k]$, $[\lambda_1, \lambda_2, \ldots \lambda_k]$ are the first k eigenvalues of $L'\, M_g L$, P_k is a matrix of the first k columns of $P = L^{-1} Q$, Q is a matrix of orthonormal eigenvectors of $L' M_g L$, $L = u^{-1}$ is the Cholesky decomposition of $M_e = u'u$, $L' M_e L = I$, I is an identity matrix, and $[M_g - M_e]$ is the non-negative definite submatrix of $L'\, M_g L$, with k eigenvalues greater than one (Amemiya 1985). The eigenvalues of $L'\, M_g L$ are equal to the eigenvalues of M_g in the metric of M_e where $|M_g - \lambda M_e| = 0$.

Jackknife and bootstrap estimators

Delete-one jacknifing was used to estimate ρ_g and the variance of ρ_g from MANOVA, REML, or ML estimates of Σ_g. Σ_g was estimated from n jackknife samples of $(n-1)$ genotypes with the ith genotype sequentially deleted (Miller 1974). The jackknife estimate of ρ_g is

$$\hat{\rho}_{g_i} = \sum_{i=1}^{n} \rho_{\tilde{g}_i}/n, \tag{4}$$

and the jackknife variance of ρ_g is

$$\hat{\sigma}^2_{\rho_j} = \sum_{i=1}^{n} (\rho_{\tilde{g}_i} - \tilde{\rho}_{g_j})^2/[n(n-1)], \tag{5}$$

where $\rho_{\tilde{g}_i} = n\hat{\rho}_g - (n-1)\, \hat{\rho}_{g_i}$ are pseudovalues, $\hat{\rho}_g$ is the MANOVA, REML, or ML estimate from the original sample, and $\hat{\rho}_{g_i}$ is the MANOVA, REML or ML estimate from the sample with the ith genotype deleted (Miller 1974). If either of the MANOVA estimates of the genetic variances were negative, then MANOVA jackknife statistics could not be estimated because $\hat{\rho}_g$ is not defined when $\hat{\sigma}^2_{g_1} < 0$ or $\hat{\sigma}^2_{g_2} < 0$.

When MANOVA estimates from the original sample were non-negative, some of the MANOVA estimates from jackknife samples could be negative and produce unbalanced samples.

Bootstrapping was used to estimate ρ_g and the variance of ρ_g from MANOVA, REML, and ML estimates of Σ_g (Efron 1982). Bootstrap samples were drawn by randomly sampling n genotypes, with replacement, where each genotype had an equal probability mass $(1/n)$. Bootstrap statistics were estimated from 100 and 500 bootstrap samples (b). The bootstrap estimate of ρ_g is

$$\hat{\rho}_{g_B} = \sum_{i=1}^{b} \hat{\rho}_{g_i}/b, \tag{6}$$

and the bootstrap variance of ρ_g is

$$\hat{\rho}^2_{g_B} = \sum_{i=1}^{b} (\hat{\rho}_{g_i} - \hat{\rho}_{g_B})^2/(b-1), \tag{7}$$

where $\hat{\rho}_{g_i}$ is the MANOVA, REML, or ML estimate of ρ_g from the ith bootstrap sample. MANOVA bootstrap statistics could not be estimated from samples producing negative variance estimates.

The parametric estimators of Tallis (1945)-Scheinberg (1966) and Reeve (1955)-Robertson (1959) were used to estimate the variance of the MANOVA estimator of ρ_g. The estimator of the variance of ρ_g proposed by Tallis (1945) and Scheinberg (1966) is

$$\hat{\sigma}^2_{\rho_g} = \frac{8\hat{\rho}^2_g}{r^2} \cdot \left[\frac{\frac{M^2_{g_1}}{df_g} + \frac{M^2_{e_1}}{df_e}}{4\hat{\sigma}^4_{g_1}} + \frac{\frac{M^2_{g_2}}{df_g} + \frac{M^2_{e_2}}{df_e}}{4\hat{\sigma}^4_{g_2}} + \frac{(M_{g_1} M_{g_2} + M^2_{g_{12}})}{(df_g)} \right.$$

$$+ \frac{(M_{e_1} M_{e_2} + M^2_{e_{12}})}{(df_e)} - \frac{M_{g_1} M_{g_{12}}}{df_g} + \frac{M_{e_1} M_{e_{12}}}{df_e}$$

$$\left. - \frac{\frac{M_{g_2} M_{g_{12}}}{df_g} + \frac{M_{e_2} M_{e_{12}}}{df_e}}{\hat{\sigma}^2_{g_2}\, \hat{\sigma}_{g_{12}}} + \frac{\frac{M^2_{g_{12}}}{df_g} + \frac{M^2_{e_{12}}}{df_e}}{2\hat{\sigma}^2_{g_1} \sigma^2_{g_2}} \right],$$

where $df_g = n - 1$ is degrees of freedom for genotypes and $df_e = n(r-1)$ is degrees of freedom for error. The estimator of the variance of ρ_g proposed by Reeve (1955) and Robertson (1959) is

$$\hat{\sigma}^2_{\rho_g} = \left[\frac{(1 - \hat{\rho}^2_g)^2\, \hat{\sigma}_{H_1} \hat{\sigma}_{H_2}}{2H_1 H_2} \right],$$

where $\hat{\sigma}^2_{H_1} = 8H_1/nr$ is the variance of H_1 and $\hat{\sigma}^2_{H_1} = 8H_2/nr$ is the variance of H_2.

Bias-corrected percentile bootstrap confidence intervals were estimated for MANOVA, REML, and ML estimators of ρ_g (Efron 1982). A $(1-\alpha)100\%$ interval for ρ_g is

$$\{cdf^{-1}[\Phi(2z_0 - z_\alpha)], \quad cdf^{-1}[\Phi(2z_0 + z_\alpha)]\} \tag{8}$$

where $cdf(y) = Pr[\hat{\rho}_g \leqslant y]$ is the cumulative distribution function (cdf) of the bootstrap point estimate of $\rho_g(\hat{\rho}_{G_B})$, $z_0 = \Phi^{-1}[cdf(\hat{\rho}_g)]$, $z_\alpha = \Phi^{-1}(1-\alpha)$, and Φ is the cdf for a standard normal variate (Efron 1982).

We used

$$[\hat{\rho}_g - z_{(1-\alpha)}\hat{\sigma}_{\rho_g}, \quad \rho_g + z_{(1-\alpha)}\hat{\sigma}_{\rho_g}]$$

to estimate two-sided $(1-\alpha)100\%$ normal-approximation intervals for ρ_g, where $\hat{\rho}_g$ is the MANOVA estimate of ρ_g, $z_{1-\alpha}$ is a random variable from the standard normal distribution, and $\hat{\sigma}_{\rho_g}$ is the Tallis (1945)-Scheinberg (1966) or Reeve (1955)-Robertson (1959) estimate of the variance of ρ_g. We used

$$[\hat{\rho}_g - t_{(1-\alpha),(y-1)}\hat{\sigma}_{\rho_g}, \quad \rho_g + t_{(1-\alpha),(y-1)}\hat{\sigma}_{\rho_g}]$$

to estimate two-sided $(1-\alpha)100\%$ normal-approximation intervals for ρ_g, where $t_{(1-\alpha),(y-1)}$ is a random variable from the t-distribution with $y-1$ degrees of freedom, $\hat{\sigma}_{\rho_g}$ is the jackknife or bootstrap variance of ρ_g, $y=n$ for the jackknife method, and $y=b$ for the bootstrap method.

Simulations

We simulated bivariate normal observations for (1) for factorial combinations of $\rho_g = -0.9, -0.5, -0.1, 0.1, 0.5$, and 0.9, $\rho_e = 0.1, 0.5$, and 0.9, $H_1 = 0.1, 0.5$, and 0.9, $H_2 = 0.1, 0.5$, and 0.9, $n = 20$, 60, and 100, and $r = 3$, 6, and 9 (1458 combinations). We simulated 2000 samples for each combination by setting $\sigma^2_{e_1} = \sigma^2_{e_2} = 1.0$ and using $\sigma_{e_{12}} = \rho_e \sigma_{e_1} \sigma_{e_2} = \rho_{e'}$, $\sigma^2_{g_1} = H_1/[r(1.0-H_1)]$, $\sigma^2_{g_2} = H_2/[r(1.0-H_2)]$, and $\sigma_{g_{12}} = \rho_g(\sigma_{g_1}, \sigma_{g_2})$ to produce Σ_g and Σ_e. These matrices were used to generate vectors of random normal deviates for g_{1j}, g_{2j}, e_{1jk}, and e_{2jk} for (1). The simulations were done using FORTRAN-77, a Floating Point System (FPS) supercomputer (FPS-264S), and FPS FORTRAN library subroutines. Uniform random numbers were produced using the RAN function from the FPS-M64 library. Univariate normal deviates were produced using the algorithm of Kinderman (1975) and uniform random numbers were produced by the RAN function. Multivariate normal deviates were produced using the algorithm of Johnson (1987).

MANOVA, ML, and REML were used to estimate ρ_g from each simulated sample. The biases of MANOVA, REML, and ML estimators of ρ_g were estimated by

$$\bar{\rho}_g - \rho_g = s^{-1} \sum_{i=1}^{s} \hat{\rho}_{g_i} - \rho_g, \tag{9}$$

and the variances of MANOVA, REML, and ML estimators of ρ_g were estimated by

$$\hat{\sigma}^2_K = \sum_{i=1}^{s} (\hat{\rho}_{g_i} - \bar{\rho}_g)^2/(s-1), \tag{10}$$

where $s = 2000$ is the number of simulated samples, $\hat{\rho}_{g_i}$ is the ANOVA, REML, or ML estimate of ρ_g from the ith simulated sample, and $\bar{\rho}_g$ is the mean of $\hat{\rho}_{g_i}$ from s simulated samples.

A bias was judged to be significantly different from zero when

$$\frac{|\bar{\rho}_g - \rho_g|}{\hat{\sigma}_{\bar{\rho}_g - \rho_g}} > t_{\alpha,(s-1)},$$

where $\hat{\sigma}_{\bar{\rho}_g - \rho_g}$ is the variance of the bias estimated from s simulated samples, $t_{\alpha,(s-1)}$ is a random variable from the t-distribution with $(s-1)$ degrees of freedom, and α is the probability of a Type-I error. This test was done using $\alpha = 0.05$. The biases of jackknife and bootstrap estimators of ρ_g were estimated using (9) by substituting (4) or (6) for $\hat{\rho}_G$ as necessary. The probability of negative estimates of $\sigma^2_{G_1}$ or $\sigma^2_{G_2}$ and the probability of estimates outside the parameter space $(-1 \leqslant \rho_G \leqslant 1)$ were estimated for the MANOVA estimator (these probabilities are 0.0 for the REML and ML estimators). Parametric, jackknife, and bootstrap estimators of the variance of ρ_g were compared to (10) for each method (MANOVA, REML, or ML) since (10) estimates the known variance.

Observed coverage probabilities $(1-\hat{\alpha})$ were compared to stated coverage probabilities $(1-\alpha)$ for each interval estimation method. Observed coverage probabilities were estimated by dividing the number of interval estimates bounding ρ_g (the known correlation) by the total number of estimates. The difference between a stated and an observed coverage probability was judged to be significantly different when

$$\frac{[1-\hat{\alpha}] - [1-\alpha]}{(\alpha[1-\alpha])/s} > z_\alpha$$

where z_α is a random variable from the standard normal distribution. We used $\alpha = 0.05$ and stated coverage probabilities of 0.80 and 0.95 for these tests.

These simulations produced numerous statistics, many of which are not directly reported, but can be found in Liu (1990).

Results and discussion

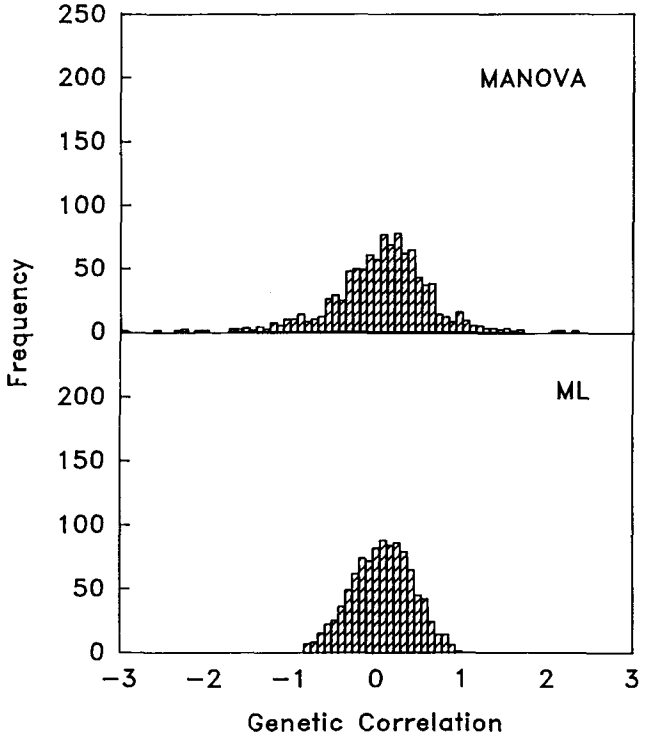
Sampling distributions

The sampling distributions for the MANOVA estimator were normal (Figs. 1–6). These distributions do not show frequencies of undefined estimates, which can be great when either heritability is low—undefined estimates are produced when one of the variances is negative. The probabilities of negative MANOVA estimates of $\sigma^2_{g_1}$ or $\sigma^2_{g_2}$ ranged from 0.00 to 0.40 (Table 1). This probability decreased as H, n, or r increased (Table 1). Means for different ρ_g and ρ_e are reported because these parameters did not affect the probability of negative estimates of $\sigma^2_{g_1}$ or $\sigma^2_{g_2}$ (Table 1).

The problem of negative estimates of $\sigma^2_{g_1}$ or $\sigma^2_{g_2}$ was greatest for low-heritability traits (Table 1). The probability ranged from 0.0 to 0.002 for high-heritability ($H_1 \geqslant 0.5$ and $H_2 \geqslant 0.5$) traits (Table 1). This problem is well known and usually leads to undefined estimates of ρ_g.

The other problem with MANOVA estimation of ρ_g is out-of-bounds estimates. The probability of out-of-bounds MANOVA estimates ($\hat{\rho}_g < -1$ or $\hat{\rho}_g > 1$) ranged from 0.0 to 0.099 (Table 2). One-hundred percent of the

Fig. 1 Sampling distributions of MANOVA and ML estimates of ρ_g for $\rho_g = 0.1$, $\rho_e = 0.1$, $H_1 = 0.1$, $H_2 = 0.1$, $n = 60$, and $r = 6$



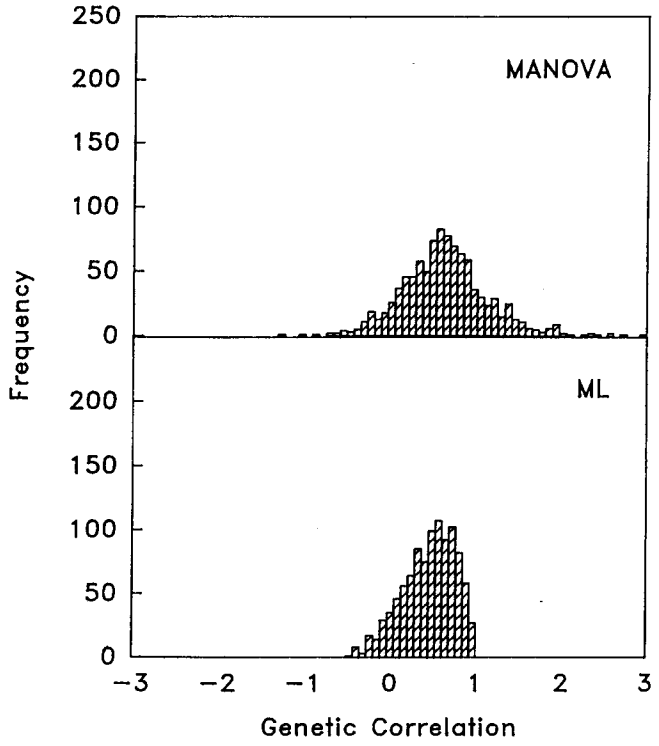


Fig. 2 Sampling distributions of MANOVA and ML estimates of ρ_g for $\rho_g = 0.5$, $\rho_e = 0.1$, $H_1 = 0.1$, $H_2 = 0.1$, $n = 60$, and $r = 6$

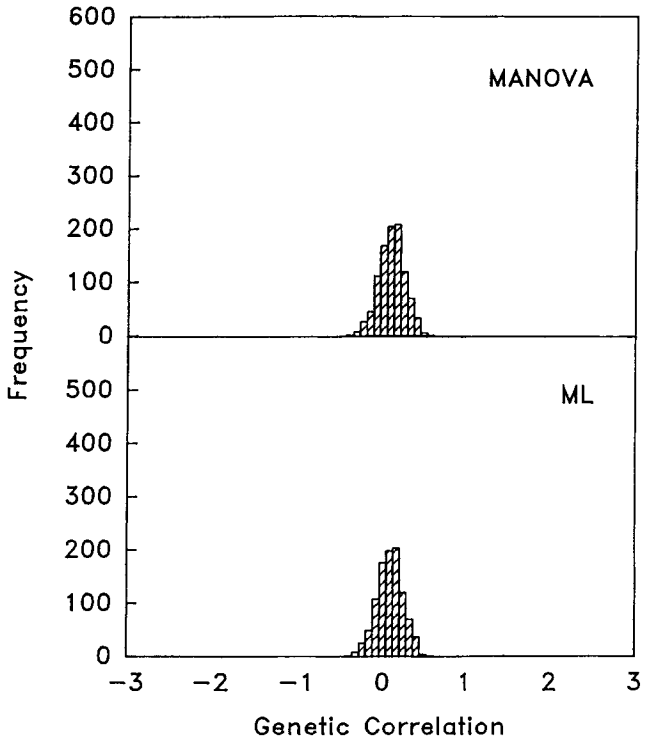


Fig. 4 Sampling distributions of MANOVA and ML estimates of ρ_g for $\rho_g = 0.1$, $\rho_e = 0.1$, $H_1 = 0.5$, $H_2 = 0.5$, $n = 60$, and $r = 6$

Fig. 3 Sampling distributions of MANOVA and ML estimates of ρ_g for $\rho_g = 0.9$, $\rho_e = 0.1$, $H_1 = 0.1$, $H_2 = 0.1$, $n = 60$, and $r = 6$

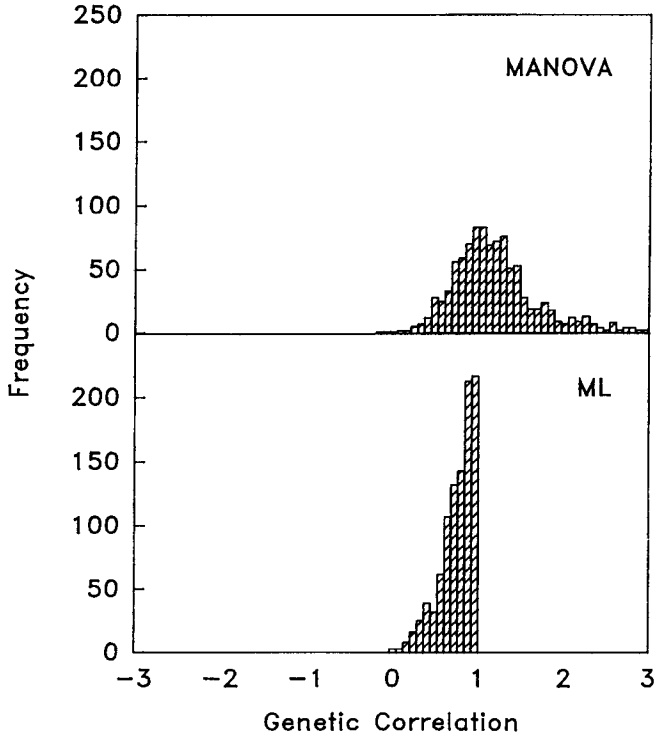
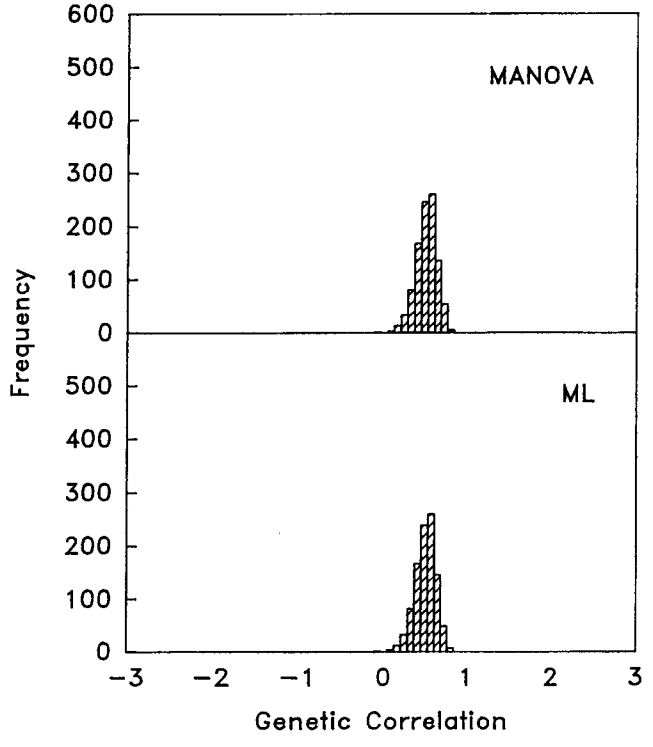


Fig. 5 Sampling distributions of MANOVA and ML estimates of ρ_g for $\rho_g = 0.5$, $\rho_e = 0.1$, $H_1 = 0.5$, $H_2 = 0.5$, $n = 60$, and $r = 6$



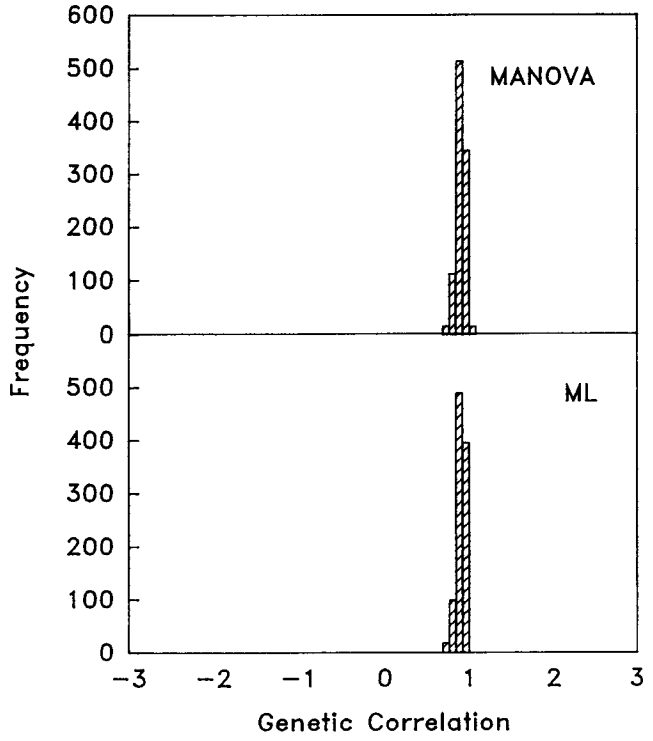


Fig. 6 Sampling distributions of MANOVA and ML estimates of ρ_g for $\rho_g = 0.9$, $\rho_e = 0.1$, $H_1 = 0.5$, $H_2 = 0.5$, $n = 60$, and $r = 6$

Table 1 Probabilities of negative MANOVA estimates of $\sigma^2_{g_1}$ or $\sigma^2_{g_2}$ for $H_1 = 0.1$ and different H_2, ρ_g, ρ_e, n, and r. The probabilities shown are means for different ρ_g and ρ_e.

H_1	H_2	n	r		
			3	6	9
0.1	0.1	20	0.402	0.185	0.077
		60	0.195	0.017	0.001
		100	0.095	0.002	0.000
	0.5	20	0.255	0.103	0.043
		60	0.110	0.008	0.000
		100	0.055	0.001	0.000
	0.9	20	0.252	0.107	0.042
		60	0.111	0.010	0.001
		100	0.055	0.001	0.000

estimates for $H_1 \geqslant 0.5$ and $H_2 \geqslant 0.5$ fell within the parameter space. Out-of-bounds estimates were only a problem when the heritability for at least one of the traits was low (Figs. 1–6 and Table 2). This probability was greatest (0.099) for $H_1 = 0.1$, $H_2 = 0.1$, $\rho_G = 0.9$, $n = 20$, and $r = 3$(Table 2). The probability of out-of-bounds estimates decreased as H, n, and r increased (Table 2). The probability of observing $\hat{\rho}_g < -1$ increased as ρ_g decreased, whereas the probability of observing $\hat{\rho}_g > 1$ increased as ρ_g increased (Table 2).

The MANOVA estimate of Σ_g is non-positive definite (NPD) when either variance is negative. Hill and Thompson (1978) showed that the probabilities of NPD MANOVA estimates of Σ_g can be great when ρ_g is high "even when neither of the heritabilities is small"; however, this probability was close to 0.0 or 0.0 when H_1 and H_2 were greater than, or equal to, 0.5 for different n, r, ρ_g, and ρ_e (Tables 1 and 2). This problem can be eliminated by using ML or REML estimators because negative and out-of-bounds ρ_g estimates are not produced with either of these methods (Amemiya 1985; Rao and Kleffe 1988).

The sampling distributions for the ML estimator were normal for some $\rho_g, \rho_e, H_1, H_2, n$ and r, as shown by the distributions for $\rho_g = 0.1$ (Figs. 1 and 4), but skewed for other $\rho_g, \rho_e, H_1, H_2, n$ and r as shown by the distributions for $\rho_g = 0.5$ and $\rho_g = 0.9$ (Figs. 2 and 5). The distributions for $\rho_g = -0.9$ were right-skewed (data not shown). Sampling distributions for the REML estimator of ρ_g were close to those of the ML estimator of ρ_g (data not shown).

Biases

The MANOVA, REML, and ML estimators of ρ_g were biased for many H, n, r, ρ_g, and ρ_e (Table 3). The bias of the ML estimator was greater than the bias of MANOVA and REML estimators for most H, n, r, and ρ_g. Differences between the estimators tended to decrease as H, n, and r increased (Table 3). Bias always decreased as r increased. Bias did not decrease as n increased for every H, r, and ρ_g; however, bias always decreased as the total number of observations ($n \cdot r$) increased (Table 3). Although the bias was fairly substantial (|0.1| to |0.2|) for some H, n, r, and ρ_g, the bias alone is usually not substantial enough to produce seriously misleading estimates and is minor compared to the sampling error of ρ_g for many H, n, and r (Table 4).

The ML estimator of σ^2_G is biased (Swallow and Monahan 1984). Swallow and Monahan (1984) produced bias-corrected ML estimates of the between-class variance by using the multiplier $(n-1)/n$. The bias-corrected ML estimator of ρ_g, however, is equal to the uncorrected ML estimator of ρ_g because the multiplier is applied to the numerator and denominator of $\hat{\rho}_g$ and so cancels. The bootstrap and jackknife estimators of ρ_g were no less biased than the usual estimators (data not shown). These estimators, at least the implementations we tried, cannot be used to bias-correct any of the ρ_g estimators tested.

Variances

We estimated the "known" variance of the MANOVA, REML, and ML estimators of $\rho_g(\hat{\sigma}^2_K)$ from the simulated samples because there was no parametric way to estimate the variance of ρ_g– the known variance of ρ_g is a function of H, n, r, ρ_g and ρ_e which are known. $\hat{\sigma}^2_K$ for the REML and ML estimators were very close, so $\hat{\sigma}^2_K$ is only shown for the latter (Table 4). The variance of the MANOVA estimator was greater than the variance of

Table 2 Probabilities of MANOVA estimates of ρ_g beyond the parameter bounds ($Pr[\hat{\rho}_g < -1]$ and $Pr[\hat{\rho}_g > 1]$) for different H_1, H_2, ρ_g, ρ_e, n, and r. The probabilities shown are means for different ρ_e

H_1	H_2	ρ_g	n	r					
				3		6		9	
				$\hat{\rho}_g < -1$	$\hat{\rho}_g > 1$	$\hat{\rho}_g < -1$	$\hat{\rho}_g > 1$	$\hat{\rho}_g < -1$	$\hat{\rho}_g > 1$
0.1	0.1	0.1	20	0.047	0.030	0.037	0.009	0.024	0.004
			60	0.038	0.008	0.012	0.001	0.002	0.0
			100	0.032	0.005	0.003	0.0	0.0	0.0
		0.5	20	0.022	0.059	0.014	0.024	0.008	0.008
			60	0.013	0.022	0.002	0.002	0.0	0.0
			100	0.008	0.014	0.0	0.0	0.0	0.0
		0.9	20	0.010	0.089	0.001	0.055	0.001	0.025
			60	0.002	0.068	0.0	0.014	0.0	0.002
			100	0.0	0.047	0.0	0.003	0.0	0.0
	0.5	0.1	20	0.015	0.008	0.005	0.002	0.003	0.001
			60	0.003	0.003	0.0	0.0	0.0	0.0
			100	0.002	0.001	0.0	0.0	0.0	0.0
		0.5	20	0.005	0.022	0.001	0.009	0.0	0.004
			60	0.0	0.010	0.0	0.001	0.0	0.0
			100	0.0	0.009	0.0	0.0	0.0	0.0
		0.9	20	0.0	0.049	0.0	0.034	0.0	0.014
			60	0.0	0.039	0.0	0.010	0.0	0.0
			100	0.0	0.027	0.0	0.002	0.0	0.0
	0.9	0.1	20	0.007	0.008	0.002	0.003	0.001	0.001
			60	0.002	0.003	0.0	0.0	0.0	0.0
			100	0.0	0.0	0.0	0.0	0.0	0.0
		0.5	20	0.001	0.019	0.0	0.011	0.0	0.005
			60	0.0	0.014	0.0	0.002	0.0	0.0
			100	0.0	0.010	0.0	0.0	0.0	0.0
		0.9	20	0.0	0.052	0.0	0.039	0.0	0.020
			60	0.0	0.041	0.0	0.010	0.0	0.001
			100	0.0	0.036	0.0	0.002	0.0	0.0

the ML estimator for most H, n, r, and ρ_g (Table 4). $\hat{\sigma}^2_K$ ranged from 0.007 to 4.535 for the MANOVA estimator and from 0.005 to 0.315 for the ML estimator (Table 4). Variances of the estimators decreased and $\hat{\sigma}^2_K$ differences decreased as H, n, and r increased (Table 4).

Of the various variance estimators tested, only the bootstrap estimator of the variance of ρ_g produced accurate estimates on a consistent basis, and then only for the REML and ML estimators of ρ_g (Table 4 and Liu 1990). We only show bootstrap variance estimates for $b = 100$ (Table 4); those for $b = 500$ were slightly superior (closer to $\hat{\sigma}^2_K$) for some H, n, and r(Liu 1990), but using more than 100 bootstrap samples is not necessary for producing accurate variance estimates. The jackknife variance estimator produced accurate estimates for $H_1 = H_2 = 0.9$ only. The variances of MANOVA, REML, and ML estimators of ρ_g were overestimated by the jackknife variance of ρ_g for many H, n, and r (Table 4).

The parametric estimators of the variance of ρ_g of Tallis (1959)-Scheinberg (1966) and Reeve (1955)-Robertson (1959) overestimated the variance of ρ_g for most H, n, r, and ρ_g (data not shown). These estimators seldom produced estimates of the variance of ρ_g which were even approximately close to the known variance (Liu 1990).

The variances of MANOVA estimators of ρ_g were greater than those for ML or REML estimators; thus, although REML and ML estimators were sometimes more biased than the MANOVA estimator, their variances were substantially less than the variance of the MANOVA estimator for most H, n, and r, especially for low heritability traits and small samples sizes (Table 4). The variance of ρ_g decreased as the number of genotypes (n) and number of replications of genotypes (r) increased (Table 4). A response-surface analysis of the effects of the different variables showed that the number of replications (r) had a significantly greater effect on the variance than the number of genotypes (n). Both n and r had a significant effect on the variance of ρ_g, but the effect of increasing r was 10-fold greater than the effect of increasing n on a per experimental unit basis; the regression coefficient for the ML method for n was -0.004 and for r was -0.04 (Liu 1990).

The problem is not this simple because increasing r increases heritability, while increasing n has no effect on heritability. The effect of r on the variance of ρ_g can only be fully assessed by decreasing the heritabilities of the two traits as r increases. This was not done in our study, nor has this been done in other studies undertaken to find optimum designs for estimating genetic (between-class) variances (Anderson and Crump 1967; Thompson 1975; Gunsett et al. 1982; Swallow and Monahan 1984; McCutchan and Namkoong 1985; Cameron and Thompson 1986).The effect of r on the variance of ρ_g might be greater than we estimated. This question is still open.

Table 3 Biases of MANOVA, ML, and REML estimated from simulated samples for $H_1 = 0.1$ and various H_2, ρ_g, n and r. Biases which were significantly different from zero ($\rho = 0.05$) are marked with an asterisk

H_2	ρ_g	n	r	MANOVA	ML	REML
0.1	0.1	20	3	0.020	0.039*	0.176*
			6	−0.153	−0.027	0.018
			9	−0.140*	−0.062*	−0.044*
		60	3	−0.177*	−0.047*	0.032
			6	−0.108*	−0.060*	−0.055*
			9	−0.050*	−0.041	−0.041*
		100	3	−0.167*	−0.067*	−0.035*
			6	−0.055*	−0.045*	−0.044*
			9	−0.019*	−0.019*	−0.019*
	0.5	20	3	0.104	−0.197*	−0.102*
			6	−0.004	−0.123*	−0.107*
			9	−0.025	−0.071*	−0.069*
		60	3	0.004	−0.130*	−0.080*
			6	−0.022	−0.036*	−0.035*
			9	−0.019*	−0.020*	−0.020*
		100	3	−0.025	−0.092*	−0.063*
			6	−0.017	−0.020*	−0.019*
			9	−0.004	−0.005	−0.005
	0.9	20	3	0.171*	−0.434*	−0.420*
			6	0.188*	−0.200*	−0.203*
			9	0.106*	−0.098*	−0.096*
		60	3	0.209*	−0.238*	−0.229*
			6	0.058*	−0.040*	−0.040*
			9	0.019*	−0.013*	−0.013*
		100	3	0.139*	−0.134*	−0.132*
			6	0.034*	−0.012	−0.012*
			9	0.011*	−0.003	−0.003
0.5	0.1	20	3	−0.028	−0.029	0.167*
			6	−0.036	−0.034*	0.048*
			9	−0.031	−0.025*	0.003
		60	3	−0.022	−0.030*	0.069*
			6	−0.021*	−0.018*	−0.011
			9	−0.007	−0.007	−0.007
		100	3	−0.019	−0.025*	0.024*
			6	−0.011	−0.011	−0.008
			9	−0.006	−0.006	−0.006
	0.5	20	3	0.046	−0.163*	0.013
			6	0.037*	−0.048*	0.015
			9	0.020	−0.018	0.009
		60	3	0.049*	−0.046*	0.023
			6	0.023*	0.006	0.014*
			9	0.006	0.005	0.005
		100	3	0.058*	−0.007	0.031*
			6	0.006	0.005	0.005
			9	0.000	0.000	0.000
	0.9	20	3	0.119*	−0.318*	−0.166*
			6	0.128*	−0.129*	−0.074*
			9	0.080*	−0.052*	−0.028*
		60	3	0.140*	−0.137*	−0.080*
			6	0.061*	−0.006	−0.003
			9	0.020*	0.002	0.003
		100	3	0.120*	−0.069*	−0.045*
			6	0.028*	0.003	0.004
			9	0.008*	0.003	0.003
0.9	0.1	20	3	0.007	−0.031*	0.175*
			6	0.002	−0.010	0.086*
			9	−0.009	−0.015	0.024
		60	3	0.005	−0.010	0.095*
			6	0.000	−0.001	0.008
			9	−0.001	−0.001	0.000
		100	3	0.005	−0.003	0.048*
			6	−0.001	−0.001	0.000
			9	0.000	0.000	0.000
	0.5	20	3	0.043	−0.160*	0.050*
			6	0.078*	−0.035*	0.055*
			9	0.036*	−0.011	0.026*
		60	3	0.079*	−0.040*	0.052*
			6	0.034*	0.014*	0.025*
			9	0.009	0.007	0.009
		100	3	0.077*	0.000	0.050*
			6	0.011*	0.009*	0.010*
			9	0.004	0.004	0.004
	0.9	20	3	0.107*	−0.321*	−0.131*
			6	0.146*	−0.130*	−0.039*
			9	0.110*	−0.046*	−0.011*
		60	3	0.156*	−0.138*	−0.054*
			6	0.070*	−0.014*	−0.004
			9	0.026*	0.002	0.002
		100	3	0.133*	−0.076*	−0.031*
			6	0.034*	0.002	0.002
			9	0.011*	0.004*	0.004*

Our findings for the variance of ρ_g are the opposite of findings for the variance of the between-class variance where increasing n is more efficient than increasing r (Anderson and Crump 1967; Thompson 1975; Gunsett et al. 1982; Swallow and Monahan 1984; McCutchan and Namkoong 1985; Cameron and Thompson 1986).

Confidence intervals

Of the various confidence interval estimators tested, only the bias-corrected bootstrap confidence interval estimator produced accurate coverages, and then only on a consistent basis for the REML and ML estimators of ρ_g (Table 5). There were no marked differences in the coverages produced by the REML and ML methods, so coverages are only shown for the latter. Liu (1990) shows the REML coverages.

The coverage of the ML bootstrap interval estimator was superior to the coverage of the MANOVA bootstrap interval estimator for most H, n, r, ρ_g. The difference was acute when either heritability was low (Table 5). The MANOVA bootstrap interval estimator greatly underestimated the stated coverage when either heritability was low (0.1), but produced accurate coverages for $H_1 \geqslant 0.5$ and $H_2 \geqslant 0.5$ for many n and r. The MANOVA estimator underestimated the stated coverage for $r = 3$ samples for most H and n (Table 5). Although the ML bootstrap interval estimator sometimes underestimated the stated coverage for low heritabilities ($H_1 = 0.1$ and $H_2 = 0.1$) and small populations ($n = 20$), the observed coverage of this estimator was still much closer to the stated coverage than the observed coverage produced by the MANOVA bootstrap interval estimator (Table 5). None of the normal-approximation interval estimators tested produced accurate coverages

Table 4 Variances of MANOVA and ML estimators of $\rho_G(\hat{\sigma}_K^2)$ and means of jackknife $(\hat{\sigma}_J^2)$ and bootstrap $(\hat{\sigma}_B^2)$ variances of MANOVA and ML estimators of ρ_G estimated from simulated samples using 100 bootstrap samples ($b = 100$) for various H_1, H_2, n and r. The variances shown are means for different ρ_g and ρ_e

H_1	H_2	n	r	MANOVA			ML		
				$\hat{\sigma}_K^2$	$\hat{\sigma}_J^2$	$\hat{\sigma}_B^2$	$\hat{\sigma}_K^2$	$\hat{\sigma}_J^2$	$\hat{\sigma}_B^2$
0.1	0.1	20	3	4.198	98.591	38.915	0.315	1.974	0.219
			6	4.535	37.987	13.023	0.257	1.261	0.232
			9	1.713	8.228	17.413	0.192	0.719	0.204
		60	3	3.207	172.287	27.953	0.251	2.655	0.205
			6	0.687	8.783	1.784	0.109	0.409	0.129
			9	0.098	1.416	0.533	0.059	0.102	0.072
		100	3	1.553	100.559	7.585	0.189	2.230	0.176
			6	0.106	1.610	1.008	0.057	0.137	0.072
			9	0.039	0.267	0.071	0.030	0.036	0.034
0.1	0.5	20	3	1.018	13.101	5.612	0.234	1.299	0.199
			6	0.450	15.891	8.988	0.159	0.753	0.170
			9	0.207	1.864	2.113	0.110	0.394	0.137
		60	3	0.451	25.083	2.943	0.124	1.526	0.125
			6	0.101	17.897	0.412	0.043	0.220	0.065
			9	0.033	0.149	0.474	0.026	0.051	0.035
		100	3	0.530	883.827	1.948	0.075	1.131	0.093
			6	0.029	0.551	0.142	0.021	0.069	0.032
			9	0.019	0.019	0.052	0.014	0.016	0.017
0.1	0.9	20	3	1.043	22.119	2.607	0.180	0.838	0.150
			6	0.440	7.480	2.838	0.133	0.515	0.136
			9	0.279	6.360	3.016	0.096	0.300	0.114
		60	3	0.400	17.524	1.104	0.099	0.967	0.104
			6	0.109	2.552	1.141	0.039	0.160	0.054
			9	0.036	0.110	0.153	0.024	0.035	0.031
		100	3	0.340	76.397	1.265	0.061	0.849	0.075
			6	0.030	0.274	0.267	0.020	0.036	0.028
			9	0.016	0.018	0.027	0.014	0.015	0.015
0.5	0.5	20	3	0.097	0.319	0.626	0.069	0.112	0.081
			6	0.054	0.053	0.130	0.044	0.052	0.047
			9	0.046	0.044	0.044	0.038	0.044	0.039
		60	3	0.023	0.021	0.020	0.018	0.020	0.019
			6	0.015	0.014	0.013	0.012	0.013	0.013
			9	0.014	0.012	0.011	0.011	0.012	0.011
		100	3	0.013	0.012	0.011	0.010	0.011	0.011
			6	0.009	0.008	0.007	0.007	0.007	0.007
			9	0.008	0.007	0.006	0.006	0.007	0.006
	0.9	20	3	0.060	0.105	0.176	0.046	0.065	0.054
			6	0.043	0.044	0.042	0.035	0.042	0.036
			9	0.040	0.039	0.034	0.033	0.038	0.033
		60	3	0.016	0.015	0.014	0.013	0.014	0.013
			6	0.014	0.012	0.011	0.011	0.011	0.011
			9	0.013	0.011	0.010	0.010	0.010	0.010
		100	3	0.009	0.008	0.008	0.007	0.008	0.007
			6	0.008	0.007	0.006	0.006	0.006	0.006
			9	0.007	0.006	0.006	0.006	0.006	0.006
0.9	0.9	20	3	0.038	0.036	0.030	0.031	0.036	0.030
			6	0.036	0.034	0.028	0.030	0.034	0.028
			9	0.036	0.034	0.028	0.029	0.033	0.028
		60	3	0.012	0.010	0.009	0.010	0.010	0.009
			6	0.011	0.010	0.009	0.009	0.009	0.009
			9	0.011	0.010	0.008	0.009	0.009	0.008
		100	3	0.007	0.006	0.005	0.006	0.006	0.005
			6	0.007	0.006	0.005	0.005	0.005	0.005
			9	0.007	0.005	0.005	0.005	0.005	0.005

and most produced extremely poor coverages (Liu 1990).

Although the MANOVA bootstrap intervals were often too narrow to produce satisfactory coverages, their widths were still 2 to 5-times greater than the widths of ML bootstrap intervals when the heritability of either trait was low (0.1) (Table 6). The mean widths of the MANOVA bootstrap interval estimator ranged from 3.50 to 7.85 when the heritabilities of both traits were low (0.1) (Table 5). These widths are roughly 2 to 4-times the width (3.5–7.9) of the parameter space $(-1 \leqslant \rho_g \leqslant 1)$ (Table 1). The mean widths of the ML bootstrap interval estimator ranged form 1.25 to 1.71 when the heritabilities of both traits were low (0.1) (Table 5). These intervals spanned 62.5 to 87.5% of parameter space.

The precision of ML (or REML) estimates of ρ_g were satisfactory for many H, n, r, and ρ_g. ML bootstrap

Table 5 Coverage probabilities for 95% bias-corrected percentile bootstrap confidence intervals for MANOVA and ML estimators of ρ_g estimated from simulated samples using 500 bootstrap samples ($b = 500$) per simulated sample for various H_1, H_2, ρ_g, n, and r. The probabilities shown are means for different ρ_e. Coverage probabilities which are significantly different ($\alpha = 0.005$) from the stated coverage probability are marked with an asterisk

H_1	H_2	ρ_g	n	r					
				MANOVA			ML		
				3	6	9	3	6	9
0.1	0.1	0.1	20	0.50*	0.65*	0.76*	0.71*	0.85*	0.92*
			60	0.66*	0.83*	0.89*	0.86*	0.96*	0.95
			100	0.74*	0.87*	0.93*	0.90*	0.94	0.94
		0.5	20	0.47*	0.59*	0.70*	0.75*	0.86*	0.92*
			60	0.63*	0.80*	0.87*	0.88*	0.95	0.95
			100	0.70*	0.87*	0.92*	0.92*	0.95	0.94
		0.9	20	0.43*	0.49*	0.53*	0.79*	0.92*	0.95
			60	0.56*	0.68*	0.76*	0.92*	0.97*	0.96*
			100	0.59*	0.77*	0.85*	0.96	0.97*	0.97*
	0.5	0.1	20	0.67*	0.77*	0.83*	0.84*	0.94	0.95
			60	0.81*	0.88*	0.91*	0.92*	0.94	0.93
			100	0.84*	0.91*	0.93*	0.93	0.94	0.93
		0.5	20	0.60*	0.72*	0.77*	0.85*	0.94	0.95
			60	0.75*	0.83*	0.90*	0.93	0.95	0.94
			100	0.77*	0.89*	0.93*	0.95	0.94	0.94
		0.9	20	0.53*	0.57*	0.60*	0.87*	0.97*	0.96*
			60	0.62*	0.71*	0.78*	0.95	0.97*	0.97*
			100	0.65*	0.81*	0.84*	0.96*	0.98*	0.97*
	0.9	0.1	20	0.69*	0.78*	0.81*	0.83*	0.93*	0.94
			60	0.81*	0.89*	0.93*	0.92*	0.94	0.94
			100	0.86*	0.91*	0.94	0.94	0.93*	0.94
		0.5	20	0.65*	0.71*	0.77*	0.83*	0.93*	0.96
			60	0.74*	0.84*	0.88*	0.94	0.96*	0.93
			100	0.77*	0.88*	0.93*	0.96*	0.95	0.94
		0.9	20	0.57*	0.56*	0.58*	0.90*	0.96*	0.97*
			60	0.61*	0.71*	0.77*	0.96*	0.97*	0.97*
			100	0.67*	0.76*	0.84*	0.97*	0.97*	0.97*
0.5	0.5	0.1	20	0.88*	0.91*	0.93*	0.93	0.92	0.93*
			60	0.94	0.93	0.94	0.94	0.93	0.94
			100	0.94	0.94	0.94	0.94	0.94	0.94
		0.5	20	0.87*	0.93*	0.93	0.94	0.93	0.94
			60	0.93*	0.93*	0.94	0.93*	0.93*	0.94
			100	0.93	0.94	0.94	0.93	0.94	0.94
		0.9	20	0.69*	0.82*	0.87*	0.94	0.93*	0.92*
			60	0.89*	0.91*	0.92*	0.94	0.93*	0.93*
			100	0.90*	0.93	0.93*	0.94	0.94	0.93*
	0.9	0.1	20	0.91*	0.94	0.93*	0.94	0.94	0.93*
			60	0.94	0.95	0.93*	0.94	0.95	0.93*
			100	0.93*	0.95	0.95	0.93*	0.95	0.95
		0.5	20	0.89*	0.92*	0.91*	0.93*	0.92*	0.91*
			60	0.93	0.95	0.94	0.93	0.95	0.94
			100	0.93*	0.94	0.94	0.93*	0.94	0.94
		0.9	20	0.74*	0.85*	0.89*	0.93	0.93*	0.92*
			60	0.88*	0.91*	0.94	0.93*	0.91*	0.94
			100	0.92*	0.93*	0.93	0.93	0.93*	0.93
0.9	0.9	0.1	20	0.95	0.93	0.94	0.95	0.93	0.94
			60	0.95	0.94	0.94	0.95	0.94	0.94
			100	0.94	0.95	0.94	0.94	0.95	0.94
		0.5	20	0.93	0.93	0.94	0.93	0.93	0.94
			60	0.94	0.94	0.93	0.94	0.94	0.93
			100	0.93	0.95	0.94	0.93	0.95	0.94
		0.9	20	0.91*	0.91*	0.91*	0.91*	0.91*	0.91*
			60	0.92*	0.94	0.93	0.92*	0.94	0.93
			100	0.93*	0.94	0.94	0.93*	0.94	0.94

interval estimates covered anywhere from 5 to 25% of the parameter space for $n = 100$ and moderate heritabilities (0.5.) (Table 5). ML interval estimates were narrower than MANOVA interval for every n, r, and ρ_g for $H_1 < 0.9$ or $H_2 < 0.9$. The widths of MANOVA, REML, and ML bootstrap interval estimates decreased as H, n, and r increased (Table 5). Heritability had the greatest effect on interval length.

MANOVA, although widely used to estimate genetic correlations, has some serious drawbacks. First, MANOVA produces negative variance (and heritability) estimates and non-positive definite estimates of genetic variance-covariance matrices (Figs. 1–6; Tables 1–2; Searle 1970; Hill and Thompson 1978). These translate into undefined and out-of-bounds estimates of ρ_g. Calvin and Dykstra (1991) described a least-squares

Table 6 Lengths of 95% bias-corrected percentile bootstrap intervals for MANOVA and ML estimators of ρ_g estimated from simulated samples using 500 bootstrap samples ($b = 500$) for various H_1, H_2, ρ_g, n, and r. The lengths shown are means for different ρ_e

H_1	H_2	ρ_g	n	r					
				MANOVA			ML		
				3	6	9	3	6	9
0.1	0.1	0.1	20	7.85	5.92	4.43	1.69	1.75	1.70
			60	5.75	2.46	1.46	1.66	1.44	1.19
			100	4.20	1.49	0.91	1.57	1.17	0.88
		0.5	20	7.24	5.47	3.69	1.71	1.67	1.59
			60	4.81	2.14	1.26	1.59	1.29	1.01
			100	3.50	1.27	0.76	1.47	1.00	0.73
		0.9	20	6.95	4.98	3.33	1.64	1.52	1.29
			60	4.87	2.22	1.09	1.43	0.94	0.56
			100	3.81	1.26	0.59	1.25	0.60	0.34
	0.5	0.1	20	4.03	2.68	2.00	1.64	1.59	1.47
			60	2.01	1.15	0.86	1.36	1.07	0.85
			100	1.51	0.80	0.61	1.16	0.78	0.61
		0.5	20	3.58	2.49	1.90	1.62	1.50	1.36
			60	1.96	1.14	0.76	1.29	0.94	0.71
			100	1.45	0.73	0.52	1.08	0.68	0.52
		0.9	20	3.61	2.48	1.83	1.54	1.31	1.08
			60	2.33	1.22	0.67	1.16	0.64	0.37
			100	1.96	0.73	0.39	0.94	0.38	0.24
	0.9	0.1	20	2.84	2.29	1.86	1.44	1.42	1.35
			60	1.75	1.05	0.82	1.20	0.96	0.80
			100	1.22	0.74	0.57	1.00	0.72	0.57
		0.5	20	2.85	2.26	1.78	1.40	1.32	1.22
			60	1.92	1.08	0.74	1.14	0.87	0.69
			100	1.48	0.73	0.50	0.98	0.65	0.50
		0.9	20	3.16	2.54	1.91	1.33	1.16	0.97
			60	2.46	1.24	0.70	1.07	0.60	0.37
			100	2.01	0.78	0.39	0.86	0.38	0.23
0.5	0.5	0.1	20	1.55	1.07	0.97	1.28	1.05	0.97
			60	0.68	0.58	0.54	0.68	0.58	0.54
			100	0.52	0.44	0.42	0.52	0.44	0.42
		0.5	20	1.29	0.89	0.79	1.10	0.88	0.79
			60	0.55	0.46	0.42	0.55	0.46	0.42
			100	0.41	0.35	0.33	0.41	0.35	0.33
		0.9	20	0.91	0.40	0.30	0.56	0.31	0.27
			60	0.27	0.17	0.15	0.23	0.17	0.14
			100	0.20	0.13	0.11	0.18	0.13	0.11
	0.9	0.1	20	1.13	0.94	0.90	1.08	0.94	0.90
			60	0.58	0.53	0.51	0.58	0.53	0.51
			100	0.45	0.41	0.40	0.45	0.41	0.40
		0.5	20	0.96	0.76	0.73	0.90	0.76	0.73
			60	0.47	0.42	0.41	0.47	0.42	0.41
			100	0.36	0.32	0.31	0.36	0.32	0.31
		0.9	20	0.58	0.32	0.26	0.40	0.27	0.25
			60	0.20	0.14	0.13	0.18	0.14	0.13
			100	0.15	0.11	0.09	0.14	0.11	0.09
0.9	0.9	0.1	20	0.87	0.84	0.84	0.87	0.84	0.84
			60	0.50	0.49	0.49	0.50	0.49	0.49
			100	0.39	0.38	0.38	0.39	0.38	0.38
		0.5	20	0.69	0.68	0.67	0.69	0.68	0.67
			60	0.39	0.38	0.38	0.39	0.38	0.38
			100	0.31	0.30	0.29	0.31	0.30	0.29
		0.9	20	0.23	0.21	0.20	0.23	0.21	0.20
			60	0.12	0.11	0.10	0.12	0.11	0.10
			100	0.09	0.08	0.08	0.09	0.08	0.08

(MANOVA) method for producing non-negative definite estimates of variance-covariance matrices, so the problem is not insurmountable. Second, variances of the MANOVA estimator are greater than variances of the REML and ML estimators for most H, n, r, and ρ_g (Table 4). The latter are more precise. Third, coverages of REML and ML bootstrap interval estimators of ρ_g are, on the whole, more accurate than coverages of MANOVA bootstrap interval estimators (Table 5), especially when heritabilities are low or moderately low, which is when the precision is worst and efficiency is most important. Fourth, REML and ML produce narrower confidence intervals (are more precise) than MANOVA (Table 6).

The biases of the REML and ML estimators of ρ_g are slightly greater than those of the MANOVA estimator of ρ_g for some H, n, and r (Table 3); however, the bias difference is negligible compared to the precision difference. Despite the superiority of the REML and ML estimators, their practical use has been limited by the lack of software with multivariate REML and ML routines. Multivariate REML and ML estimation has been addressed by several authors (Thompson 1973; Smith 1985; Meyer 1991). The proposed solutions are not yet found in commercial software, which has limited the use of REML and ML for estimating covariances and genetic correlations.

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