Estimation of the Proportion of Genetic Variation Accounted for by DNA Tests

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Abstract

An increasingly relevant question in evaluating commercial DNA tests is "What proportion of the additive genetic variation in the target trait is accounted for by the test?" Therefore, several estimators of this quantity were evaluated by simulation of a population of 1000 animals with 100 sires, each with 10 progeny. Three heritabilities (0.1, 0.3, and 0.5) of the target trait and four proportions of genetic variation (0.04, 0.16, 0.36, and 0.64) accounted for by the molecular breeding value (**MBV**) for the DNA test were simulated. The first estimator evaluated is the reduction in estimated sire variance ($\hat{\boldsymbol{R}}_{\boldsymbol{g_{RV}}}^{\boldsymbol{2}}$) when the MBV is added as a fixed covariate to a single-trait model for the target trait divided by the sire variance from the model without the MBV. The second estimator is based on the regression of phenotype on MBV ($\hat{\boldsymbol{R}}_{\boldsymbol{g_{RPM}}}^{\boldsymbol{2}}$) from a single trait sire model in which the MBV is a fixed covariate (this is the model that has been standard in independent validations since DNA tests began being reported as MBV). This estimator is computed as $\hat{R}_{g_{RPM}}^{2} \cong \frac{\hat{b}^2 \hat{\sigma}^2_{p_m}}{\hat{\sigma}^2_{g_y} \hat{h}^2_{g_m}}$ where $\hat{b}$ is the regression of the target phenotype on MBV, $\hat{\sigma}^2_{p_m}$ is the phenotypic variance of the MBV, $\hat{h}^2_{g_m}$ is the heritability of the MBV, and $\hat{\sigma}^2_{g_y}$ is the additive genetic variance of the target trait. The third estimator is the restricted maximum likelihood (**REML**) estimate of additive genetic correlation squared ($\hat{\boldsymbol{R}}_{\boldsymbol{g_{MT}}}^{\boldsymbol{2}}$) in a two-trait animal model for the target trait and the MBV (as the second trait). In this case, the only fixed effect in the model for MBV is a mean. The standard error of $\hat{R}_{g_{MT}}^{2}$ was computed by multiplying the standard error of the genetic correlation by twice the genetic correlation. The mean of $\hat{R}_{g_{MT}}^{2}$ tended to be closer to the simulated values than $\hat{R}_{g_{RV}}^{2}$ and $\hat{R}_{g_{RPM}}^{2}$, although all three estimators performed reasonably for most parameter sets. The standard deviations of estimates among replicates of $\hat{R}_{g_{MT}}^{2}$ were generally smaller than $\hat{R}_{g_{RV}}^{2}$ and $\hat{R}_{g_{RPM}}^{2}$ and, at low heritability, were much smaller. All three estimators can produce erratic results in replicates in which the estimate of the additive variance approaches zero. Data sets in which the estimated heritability is much lower than expected should be considered inadequate for estimating the proportion of additive variation. The $\hat{R}_{g_{RV}}^{2}$ estimator can produce negative estimates and the $\hat{R}_{g_{RV}}^{2}$ and $\hat{R}_{g_{RPM}}^{2}$ estimators can produce estimates > 1 of the proportion of additive variance explained. The $\hat{R}_{g_{MT}}^{2}$ estimator has the advantage of producing estimates within the

parameter space. The computed standard errors of $\hat{R}_{g_{MT}}^2$ were similar to the standard deviation of the estimates. This property is another advantage of $\hat{R}_{g_{MT}}^2$ over $\hat{R}_{g_{RV}}^2$ and $\hat{R}_{g_{RPM}}^2$, for which empirical methods for computing standard errors are not obvious. It is recommended that the $\hat{R}_{g_{MT}}^2$ estimator be used for estimating the proportion of additive variation explained by a DNA test and that it be referred to simply as $\hat{R}_g^2$. Similar estimators of the proportion of phenotypic variance explained by DNA tests for application in marker-assisted management (**MAM**) will also be explored. Practical considerations in the application of these statistics are discussed.

Introduction

The proportion of additive genetic variation accounted for by a DNA test is a useful metric with which to quantitatively evaluate the merit of commercial DNA tests for marker-assisted selection (**MAS**) of seedstock. Until now, the estimation of this statistic has been considered a difficult problem.

We assume throughout that DNA test results will be presented in the form of molecular breeding values (**MBV**). They are continuous values intended to predict the breeding values of animals based only on the DNA test results. They are typically expressed in units of the trait and assumed to be scaled equivalent to twice the EPD. However, in practice, they are often scaled differently. There should be an MBV for each trait that a DNA test is capable of predicting. Most current commercial DNA tests for quantitative traits in beef cattle are expressed as MBV (or closely related values), although some companies may use different names for them.

Here we describe a theoretically desirable estimator that should be computationally feasible for data sets of the size we are likely to be able to use for estimation. It is computed from a model that has desirable properties for the inclusion of MBV in the national cattle evaluation (**NCE**) system. This estimator of the proportion of additive genetic variation due to MBV is directly related to the variances and covariances that are required to incorporate MBV into NCE as described by Kachman (2008) and applied by MacNeil *et al*. (2009). Inclusion of MBV into NCE in lieu of genotypes is necessary because NCE does not have access to individual SNP genotypes associated with the commercialized tests.

We will also describe several other estimators of proportions of variation derived from simpler models and compare their performance on simulated data. We will also examine some of the shortcomings of these estimators and the effects of some basic assumptions on the comparison.

*Marker Assisted Management (**MAM**)*

DNA tests have at least as much potential prediction of genetic merit of commercial cattle for application in MAM as they have for the application of MAS in breeding cattle. Two fundamental differences between these applications exist. First, in MAS, the objective is to improve breeding value (the additive component of genetic merit) and therefore MBV for application in MAS should not include the non-additive components (dominance and epistasis) of total genetic merit. However, for applications in MAM, the objective is to predict phenotypes; consequently, the DNA test should

predict total genetic merit (including the non-additive as well as additive components). Therefore, the way in which DNA test results are computed should be different between MAS and MAM.

The term molecular breeding value is specific to the application of DNA testing to selection. The corresponding term which is more appropriate for MAM is molecular genetic value (**MGV**), which is a continuous value, intended to predict the total genetic merit of animals based only on the DNA test results. As for MBV, MGV would ideally be expressed in the units of the trait they are intended to predict. The proportion of variation that is relevant in MAM is the proportion of phenotypic variation that is accounted for by the MGV.

The second fundamental difference between MAS and MAM is that, in MAS, pedigree and breed composition are typically known. Consequently, it is often feasible to statistically partition the additive genetic from the residual components of phenotype. (The residual consists of the non-additive genetic and non-genetic components of the phenotype, which are usually assumed to be confounded with one another. Pedigree structures are typically not adequate to effectively partition these two components.) A prerequisite for a population to be suitable for estimation of the proportion of additive genetic variance due to an MBV is that its pedigree be adequate to partition the additive genetic from the residual components.

However, for application in MAM, pedigree and breed composition are often unknown. Fortunately, there is no need to partition the additive genetic component from the remainder of phenotype for MAM.

Objectives and Intended Audience

This paper is more technical than is typical for BIF proceedings. It is not intended for the entire audience of the BIF convention. It is included here because its development occurred after the last Genetic Prediction Workshop (**GPW**) and it provides the basis for standardizing statistical procedures necessary to implement recommendations made at the last GPW (Moser, 2008) regarding how independent evaluations of DNA tests should be conducted and reported.

It is intended that these new statistics will enhance (in the shorter term) and eventually replace (in the longer term) the "validation" component (Van Eenennaam *et al*., 2007) of the National Beef Cattle Evaluation Consortium's (NBCEC's) third party evaluation of DNA tests. This process is in transition towards greater emphasis on estimation of the (co)variances required to incorporate DNA tests into NCE and providing information that is more useful to customers of the technology. As such, the primary objective of this paper is to characterize statistics that will improve the ability of cattle producers to make informed decisions regarding the purchase of DNA tests for MAS and MAM. A secondary objective is to discuss practical considerations in the application of these statistics, specifically in the NBCEC third party evaluation of DNA tests as described by Van Eenennaam *et al*. (2009). The first example of results reported in this way is at http://www.beefcrc.com.au/Aus-Beef-DNA-results (accessed 4/15/09). Consequently, this paper is intended to serve as reference material for recommendations in the BIF Guidelines.

Notation

The MBV and MGV will be referred to collectively as molecular values (**MV**). We will use MBV to refer to an MV that is intended to be used in MAS. Our position is that MBV should include only additive genetic effects (have heritability close to one). Nonetheless, only for the purposes of this paper, we admit the possibility that MBV could be contaminated with non-additive genetic components solely for the purpose of evaluating their effects on the various estimators to be considered. Similarly, we will use MGV to refer to an MV that is intended to be used in MAM, regardless of whether it includes non-additive genetic effects or not.

We will derive estimators of proportions of variance from a two-trait animal model with the MV and observed phenotype included as correlated traits. The models for observed and MV traits will each include an animal effect (with variance structure proportional to the numerator relationship matrix), appropriate fixed effects, and a residual (with variance proportional to the identity matrix).

This approach is readily extended to models with multiple observed traits and multiple MV traits. In practice the method should be applied to models with multiple traits of both types. The use of a two-trait model here is for notational simplicity only.

The two-trait model is represented as:

$$\begin{bmatrix}\mathbf{y}\\ \mathbf{m}\end{bmatrix} = \begin{bmatrix}\mathbf{X}_y & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_m\end{bmatrix}\begin{bmatrix}\beta_y\\ \beta_m\end{bmatrix} + \begin{bmatrix}\mathbf{Z}_y & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_m\end{bmatrix}\begin{bmatrix}\mathbf{u}_y\\ \mathbf{u}_m\end{bmatrix} + \begin{bmatrix}\mathbf{e}_y\\ \mathbf{e}_m\end{bmatrix}$$

$$\begin{bmatrix}\mathbf{u}_y\\ \mathbf{u}_m\\ \mathbf{e}_y\\ \mathbf{e}_m\end{bmatrix} \sim \left(\begin{bmatrix}\mathbf{0}\\ \mathbf{0}\\ \mathbf{0}\\ \mathbf{0}\end{bmatrix}, \begin{bmatrix}\sigma^2_{g_y}\mathbf{A} & \sigma_{g_{ym}}\mathbf{A} & \mathbf{0} & \mathbf{0}\\ \sigma_{g_{ym}}\mathbf{A} & \sigma^2_{g_m}\mathbf{A} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \sigma^2_{r_y}\mathbf{I} & \sigma_{r_{ym}}\mathbf{I}\\ \mathbf{0} & \mathbf{0} & \sigma_{r_{ym}}\mathbf{I} & \sigma^2_{r_m}\mathbf{I}\end{bmatrix}\right)$$

where:

y = a vector of phenotypes for the observed trait,
m = a vector of MV,
β_y = fixed effects appropriate to **y**,
β_m = fixed effects appropriate to **m**,
$\mathbf{u}_y$, $\mathbf{u}_m$ = additive genetic components of **y** and **m**, respectively,
$\mathbf{e}_y$, $\mathbf{e}_m$ = residuals of **y** and **m**, respectively,
$\mathbf{X}_y$, $\mathbf{X}_m$, $\mathbf{Z}_y$, $\mathbf{Z}_m$ are design matrices relating β_y, β_m, $\mathbf{u}_y$, and $\mathbf{u}_m$ to their respective observations,
A = the numerator relationship matrix,
$\sigma^2_{g_y}$ = additive genetic variance of **y**,
$\sigma_{g_{ym}}$ = additive genetic covariance between **m** and **y**,
$\sigma^2_{g_m}$ = additive genetic variance of **m**,
$\sigma^2_{r_y}$ = residual variance of **y**,
$\sigma_{r_{ym}}$ = residual covariance between **m** and **y**,
$\sigma^2_{r_m}$ = residual variance of **m**.

Additionally,

$\sigma^2_{g_y|g_m}$ = additive genetic variance of **y**, conditional on the additive genetic effect of **m**.
$\sigma^2_{g_y} - \sigma^2_{g_y|g_m}$ = additive genetic variance of **y** that is accounted for by **m**.

$R_g^2 = \frac{\sigma^2_{g_y} - \sigma^2_{g_y|g_m}}{\sigma^2_{g_y}}$ = proportion of additive genetic variance accounted for by additive genetic effect of **m**.

$\sigma^2_{p_y} = \sigma^2_{g_y} + \sigma^2_{r_y}$ = phenotypic variance of **y**,

$\sigma_{p_{ym}} = \sigma_{g_{ym}} + \sigma_{r_{ym}}$ = phenotypic covariance between **m** and **y**,

$\sigma^2_{p_m} = \sigma^2_{g_m} + \sigma^2_{r_m}$ = phenotypic variance of **m**,

$r_g = \frac{\sigma_{g_{ym}}}{\sigma_{g_y}\sigma_{g_m}}$ = additive genetic correlation between **m** and **y**,

$r_p = \frac{\sigma_{p_{ym}}}{\sigma_{p_y}\sigma_{p_m}}$ = phenotypic correlation between **m** and **y**,

$h^2_{g_y} = \sigma^2_{g_y}/\sigma^2_{p_y}$ = the narrow sense heritability of **y**,

$h^2_{g_m} = \sigma^2_{g_m}/\sigma^2_{p_m}$ = the narrow sense heritability of **m**,

$h^2_{t_m} = (\sigma^2_{g_m} + \sigma^2_{na_m})/\sigma^2_{p_m}$ = the broad sense heritability of **m**.

The above equations represent the additive model. Notation incorporating non-additive variation includes

$\sigma^2_{na_m}$ = the non-additive genetic variance of **m**,

$\sigma_{na_{ym}}$ = the non-additive genetic covariance between **m** and **y**,

$\sigma^2_{na_y}$ = the non-additive genetic variance of **y**,

$\sigma^2_{na_y|na_m}$ = non-additive genetic variance of **y**, conditional on the non-additive genetic effect of **m**,

$\sigma^2_{t_y} = \sigma^2_{g_y} + \sigma^2_{na_y}$ = total genetic variance of **y**,

$\sigma_{t_{ym}} = \sigma_{g_{ym}} + \sigma_{na_{ym}}$ = total genetic covariance between **m** and **y**,

$\sigma^2_{t_m} = \sigma^2_{g_m} + \sigma^2_{na_m}$ = total genetic variance of **m**,

$\sigma^2_{t_y|t_m}$ = total genetic variance of **y**, conditional on the additive genetic effect of **m**.

$\sigma^2_{t_y} - \sigma^2_{t_y|t_m}$ = total genetic variance of **y** that is accounted for by **m**.

$R_p^2 = \frac{\sigma^2_{t_y} - \sigma^2_{t_y|t_m}}{\sigma^2_{p_y}}$ = proportion of phenotypic variance accounted for by total genetic effects of **m**.

Analyses used for the independent validation of DNA tests have typically been conducted using a single trait sire model for the target trait in which the MV is included as a covariate. For one of the estimators to be considered, it is also useful to use a reduced model in which the MV covariate is dropped from the full model. These models can be represented as:

$$\mathbf{y} = \mathbf{X}_y\beta_y + \mathbf{m}b + \mathbf{Z}_s\mathbf{s}_1 + \mathbf{e}_1 \text{ (full)}$$

$$\mathbf{y} = \mathbf{X}_y\beta_y + \mathbf{Z}_s\mathbf{s}_0 + \mathbf{e}_0 \text{ (reduced)}$$

$$\begin{bmatrix}\mathbf{s}_1\\ \mathbf{e}_1\end{bmatrix} \sim \left(\begin{bmatrix}\mathbf{0}\\ \mathbf{0}\end{bmatrix}, \begin{bmatrix}\sigma^2_{s_1}\mathbf{A}_s & \mathbf{0}\\ \mathbf{0} & \sigma^2_{r_1}\mathbf{I}\end{bmatrix}\right)$$

$$\begin{bmatrix}\mathbf{s}_0\\ \mathbf{e}_0\end{bmatrix} \sim \left(\begin{bmatrix}\mathbf{0}\\ \mathbf{0}\end{bmatrix}, \begin{bmatrix}\sigma^2_{s_0}\mathbf{A}_s & \mathbf{0}\\ \mathbf{0} & \sigma^2_{r_0}\mathbf{I}\end{bmatrix}\right)$$

where:

$\mathbf{s}_1$, $\mathbf{s}_0$ = random sire effects in the full or reduced models, respectively,

$\mathbf{e}_1$, $\mathbf{e}_0$ = residuals of **y** in the full or reduced models, respectively,

$\mathbf{Z}_s$ is a design matrix relating sires to observations,

$\mathbf{A}_s$ = the numerator relationship matrix of the sires,
$\sigma^2_{s_1}$, $\sigma^2_{s_0}$ = sire variance in the full or reduced models, respectively,
$\sigma^2_{r_1}$, $\sigma^2_{r_0}$ = residual variance of **y** in the full or reduced models, respectively,

Estimators

Multiple trait model for MAS

An estimator based on the multiple trait model is derived as follows: by the definition of conditional variance, $\sigma^2_{g_y|g_m} = \sigma^2_{g_y} - \frac{(\sigma_{g_{ym}})^2}{\sigma^2_{g_m}}$ and therefore,

$$R_{g_{MT}}{}^2 = \frac{\sigma^2_{g_y} - \sigma^2_{g_y|g_m}}{\sigma^2_{g_y}} = \frac{\sigma^2_{g_y} - \left(\sigma^2_{g_y} - \frac{(\sigma_{g_{ym}})^2}{\sigma^2_{g_m}}\right)}{\sigma^2_{g_y}} = \frac{(\sigma_{g_{ym}})^2}{\sigma^2_{g_y}\sigma^2_{g_m}} = (r_g)^2$$

$$\hat{R}_{g_{MT}}{}^2 = \frac{(\hat{\sigma}_{g_{ym}})^2}{\hat{\sigma}^2_{g_y}\hat{\sigma}^2_{g_m}} = (\hat{r}_g)^2$$

and $\hat{R}_{g_{MT}}{}^2 = (\hat{r}_g)^2$ is an estimator of the proportion of additive genetic variance accounted for by the MBV. This is simply the square of the additive genetic correlation between the observed and MBV traits.

If the (co)variances are estimated by REML, then the squared genetic correlation will be a REML estimate of the proportion of variation. Therefore, it has the desirable property that the estimate will be within the parameter space (between 0 and 1).

This estimator has been used for the analysis of real data in Australia (http://www.beefcrc.com.au/Aus-Beef-DNA-results, accessed 4/15/09).

The delta method (Oehlert, 1992) can then be used to obtain an approximate standard error of the squared genetic correlation by multiplying the standard error of the genetic correlation by the absolute value of the partial derivative of $(\hat{r}_g)^2$ with respect to $\hat{r}_g$. The approximate standard error of $\hat{R}_{g_{MT}}{}^2$ is obtained by multiplying the standard error of r_g by $2|\hat{r}_g|$. Using Appendix 1,

$$\text{Var}[(\hat{r}_g)^2] \cong 4(\hat{r}_g)^2 \text{ Var}[\hat{r}_g]$$

$$\text{se}[\hat{R}_{g_{MT}}{}^2] = \text{se}[(\hat{r}_g)^2] \cong 2|\hat{r}_g| \text{ se}[\hat{r}_g]$$

The se($R_g{}^2$) has the same statistical properties as other functions (correlations and heritabilities) of the estimated (co)variance parameters.

If it is assumed that the MV is a true MBV, based on a purely additive genetic model, then the narrow sense heritability of that MBV should be very high and the residual variance may be due only to laboratory (including missing genotypes), pedigree, sample identification and/or other independent errors. Thus, these small residuals should be uncorrelated with the residuals of the phenotypes. Therefore, it would be reasonable to fix the residual covariance to zero, and hence, reduce the number of parameters to be estimated by one. However, if the MV presented as an MBV includes non-additive genetic components (or if some residual variation was due to pedigree errors), this could induce a residual covariance and it may be best to estimate that covariance. Thus, there are two slightly different variations of the $\hat{R}_{g_{MT}}{}^2$ estimator that are reasonable to consider using.

Reduction in Sire Variance for MAS

The single trait sire model has typically been used in independent validations of DNA tests reported as MBV. An ad hoc estimator of the proportion of additive genetic variation due to the MBV that has been used in some cases is the reduction in sire variance (**RV**). It is based on the rationale that the reduction in the sire variance when the MBV is included in the model is equivalent to the variation accounted for by the MBV. Thus,

$$\hat{R}_{g_{RV}}{}^2 = \frac{4\hat{\sigma}^2_{s_0} - 4\hat{\sigma}^2_{s_1}}{4\hat{\sigma}^2_{s_0}} = \frac{\hat{\sigma}^2_{s_0} - \hat{\sigma}^2_{s_1}}{\hat{\sigma}^2_{s_0}}$$

because four times both the numerator and the denominator reflects the proportional reduction in additive genetic variation. This rationale should be valid whether the MV contains a non-additive genetic component or not.

Regression of Phenotype on MBV for MAS

An alternative estimator of the proportion of additive genetic variation due to the MBV, based on the regression of phenotype on MBV (**RPM**) in the full single-trait model used in independent validations can be derived. Although this model may be fit as a sire model, the notation is simpler if we reparameterize it in terms of the additive genetic variance, $\sigma^2_{g_y} = 4\sigma^2_{s_1}$, and other similar parameters defined for the multiple trait animal model.

By the definition of regression, $b = \frac{\sigma_{p_{ym}}}{\sigma^2_{p_m}}$. Thus,

$$b = \frac{\sigma_{p_{ym}}}{\sigma^2_{p_m}} = \frac{\sigma_{g_{ym}} + \sigma_{r_{ym}} + \sigma_{g_y r_m} + \sigma_{r_y g_m}}{\sigma^2_{p_m}} = \frac{\sigma_{g_{ym}} + \sigma_{r_{ym}}}{(\sigma^2_{g_m} + \sigma^2_{na_m})/h^2_{t_m}}$$

because $\sigma_{g_y r_m}$, the covariance between $\mathbf{u}_y$ and $\mathbf{e}_m$, and $\sigma_{r_y g_m}$, the covariance between $\mathbf{e}_y$ and $\mathbf{u}_m$, are both assumed to be zero as is standard in animal breeding models, even if the MBV contains some non-additive genetic effects.

In this model, $b/h^2_{t_m}$ essentially serves as a scaling factor, translating units of the observed trait to units of the MBV. It can be argued that this scaling factor should apply equally to the additive and non-additive genetic components of any MV. Thus,

$$\frac{b}{h^2_{t_m}} = \frac{\sigma_{g_{ym}} + \sigma_{na_{ym}}}{\sigma^2_{g_m} + \sigma^2_{na_m}} = \frac{\sigma_{g_{ym}}}{\sigma^2_{g_m}} = \frac{\sigma_{na_{ym}}}{\sigma^2_{na_m}}$$

$$\sigma_{g_{ym}} = \frac{\sigma^2_{g_m} b}{h^2_{t_m}}.$$

This estimator makes use of the additive variance, conditional on the additive genetic effect of the MBV, $\sigma^2_{g_y|g_m}$. The additive genetic variance of the observed trait that is accounted for by the MBV is $\sigma^2_{g_y} - \sigma^2_{g_y|g_m}$. Therefore, the proportion of additive genetic variance accounted for by the MBV is

$$R_{g_{RPM}}{}^2 = \frac{\sigma^2_{g_y} - \sigma^2_{g_y|g_m}}{\sigma^2_{g_y}} = \frac{\sigma^2_{g_y} - \left(\sigma^2_{g_y} - \frac{(\sigma_{g_{ym}})^2}{\sigma^2_{g_m}}\right)}{\sigma^2_{g_y}} = \frac{(\sigma_{g_{ym}})^2}{\sigma^2_{g_y}\sigma^2_{g_m}} = \frac{(\sigma^2_{g_m} b)^2}{\sigma^2_{g_y}\sigma^2_{g_m} (h^2_{t_m})^2}$$

$$R_{g_{RPM}}{}^2 = \frac{b^2 \sigma^2_{g_m}}{\sigma^2_{g_y} (h^2_{t_m})^2}$$

Therefore, $\hat{R}_{g_{RPM}}{}^2 = \frac{\hat{b}^2 \hat{\sigma}^2_{g_m}}{\hat{\sigma}^2_{g_y} (\hat{h}^2_{t_m})^2}$ is an estimator of the proportion of additive genetic variance accounted for by an MBV, without an assumption of additivity of the MBV. However, it does require an estimate of the broad sense heritability of the MBV. In most cases, this will be impractical to estimate. Therefore, an assumption will be required.

Broad sense heritability of an MBV should differ from one only due to pedigree, sample identification, or laboratory (including missing genotypes) errors. If these are assumed to be minimal, then it could be reasonable to approximate $h^2_{t_m} \cong 1$, in which case $\hat{R}_{g_{RPM}}{}^2 \cong \frac{\hat{b}^2 \hat{\sigma}^2_{g_m}}{\hat{\sigma}^2_{g_y}}$ is an estimator in which the approximation introduces a slight downward bias.

Alternatively, if the MBV is strictly additive, then the non-additive genetic variance of the MBV, $\sigma^2_{na_m}$, is equal to zero and consequently $h^2_{t_m} = h^2_{g_m}$. Therefore, the estimator simplifies to $\hat{R}_{g_{RPM}}{}^2 \cong \frac{\hat{b}^2 \hat{\sigma}^2_{p_m}}{\hat{\sigma}^2_{g_y} \hat{h}^2_{g_m}}$, under this approximation, which introduces an upward bias if the MV presented as an MBV actually contains a non-additive genetic component.

If the MBV are computed using a strictly additive model, then $\hat{h}^2_{g_m}$ should be very close to one, which implies that $\hat{\sigma}^2_{g_m}$ will also be very close to $\hat{\sigma}^2_{p_m}$. Therefore, in many practical cases, these approximations may be inconsequential. In any of the versions of this estimator, $\hat{b}$ and $\hat{\sigma}^2_{g_y}$ are obtained from the full single trait model whereas $\hat{h}^2_{g_m}$, $\hat{\sigma}^2_{p_m}$, and/or $\hat{\sigma}^2_{g_m}$ must be obtained from a separate single trait analysis of the MBV.

Multiple trait model for MAM

An estimator of the proportion of phenotypic variance accounted for by the MV based on the multiple trait model is derived as follows: Because the phenotypic covariance, $\sigma_{p_{ym}}$, should be due only to genetic effects, it should be equal to the total genetic covariance, $\sigma_{t_{ym}}$ ($\sigma_{p_{ym}} = \sigma_{t_{ym}}$). By the definition of conditional variance, $\sigma^2_{t_y|t_m} = \sigma^2_{t_y} - \frac{(\sigma_{t_{ym}})^2}{\sigma^2_{t_m}}$ and therefore,

$$R_{p_{MT}}{}^2 = \frac{\sigma^2_{t_y} - \sigma^2_{t_y|t_m}}{\sigma^2_{p_y}} = \frac{\sigma^2_{t_y} - \left(\sigma^2_{t_y} - \frac{(\sigma_{t_{ym}})^2}{\sigma^2_{t_m}}\right)}{\sigma^2_{p_y}} = \frac{(\sigma_{t_{ym}})^2}{\sigma^2_{p_y}\sigma^2_{t_m}} = \frac{(\sigma_{p_{ym}})^2}{\sigma^2_{p_y}\sigma^2_{p_m}h^2_{t_m}} = \frac{(r_p)^2}{h^2_{t_m}}.$$

and $\hat{R}_{p_{MT}}{}^2 = (\hat{r}_p)^2/\hat{h}^2_{t_m}$ is an estimator of the proportion of phenotypic variance accounted for by the MV. This is the square of phenotypic correlation between the observed and MV traits, divided by the broad sense heritability. In general, the broad sense heritability will not be feasible to estimate. However, it should be only slightly less than one, the difference being due to laboratory errors, missing genotypes, or sample identification

errors. Therefore, the practical solution appears to be to assume that $h^2_{t_m} = 1$, in which case, $\hat{R}_{p_{MT}}{}^2 \cong (\hat{r}_p)^2$.

The approximate standard error of $\hat{R}_p{}^2$ is obtained as:

$$se[\hat{R}_{p_{MT}}{}^2] = se[(\hat{r}_p)^2] = 2|\hat{r}_p|\ se[\hat{r}_p]$$

by substituting $\hat{r}_p$ for $\hat{r}_g$ in the derivation of $se[\hat{R}_{g_{MT}}{}^2]$.

Because this approach does not require partitioning the genetic from non-genetic effects, it could also be applied in models that do not contain genetic effects. However, because the residual covariance is a potentially important component of the phenotypic correlation, it should always be estimated in models being run for the estimation of $\hat{R}_{p_{MT}}{}^2$.

Reduction in Variance for MAM

An ad hoc estimator of the proportion of phenotypic variation due to the MV can be developed from full and reduced single trait models analogous to the $\hat{R}_{g_{RV}}{}^2$ estimator for MAS. It is based on the rationale that the reduction in the phenotypic variance when the MV is included in the model is equivalent to the total genetic variation accounted for by the MV. Thus,

$$\hat{R}_{p_{RV}}{}^2 = \frac{(\hat{\sigma}^2_{s_0} + \hat{\sigma}^2_{r_0}) - (\hat{\sigma}^2_{s_1} + \hat{\sigma}^2_{r_1})}{(\hat{\sigma}^2_{s_0} + \hat{\sigma}^2_{r_0})}.$$

This general approach could also be applied in models that do not contain sire, animal, or other genetic effects in the model.

Regression of Phenotype on MV for MAM

An estimator of the proportion of phenotypic variation due to the MV, analogous to the $\hat{R}_{g_{RPM}}{}^2$ estimator defined above can be derived. The phenotypic variance of the observed trait that is accounted for by the MV is $\sigma^2_{p_y} - \sigma^2_{p_y|p_m}$. The variance of phenotypes in the full model, conditional on the MV is $\sigma^2_{p_y|p_m} = \sigma^2_{p_y} - \frac{(\sigma_{p_{ym}})^2}{\sigma^2_{p_m}}$. By the definition of regression, $b = \frac{\sigma_{p_{ym}}}{\sigma^2_{p_m}}$, where $\sigma_{p_{ym}}$ is the phenotypic covariance between **y** and **m** in the full single trait model. Thus,

$$R_{p_{RPM}}{}^2 = \frac{\sigma^2_{p_y} - \sigma^2_{p_y|p_m}}{\sigma^2_{p_y}} = \frac{\sigma^2_{p_y} - \left(\sigma^2_{p_y} - \frac{(\sigma_{p_{ym}})^2}{\sigma^2_{p_m}}\right)}{\sigma^2_{p_y}} = \frac{(\sigma_{p_{ym}})^2}{\sigma^2_{p_y}\sigma^2_{p_m}} = \frac{b^2\,\sigma^2_{p_m}}{\sigma^2_{p_y}}$$

Therefore, $\hat{R}_{p_{RPM}}{}^2 = \frac{\hat{b}^2\,\hat{\sigma}^2_{p_m}}{\hat{\sigma}^2_{p_y}}$ is an estimator of the proportion of phenotypic genetic variance accounted for by an MV. This estimator requires relatively few assumptions although it does require an estimate of the phenotypic variance of MV from a separate analysis.

Estimation of Regression Coefficient with Single Trait Model

The regression of the phenotype on the MV is essentially a scaling factor that converts the units of the observed trait into units of the MV. Ideally, it would be equal to one, but it has been found typically to be less than one for commercial DNA tests. In the full single trait analysis, this regression is estimated directly in the analysis, along with its standard error. We will refer to it as $\hat{b}_{ST}$. Previous NBCEC validations of MBV (http://www.nbcec.org/) have been primarily based on checking for evidence that $\hat{b}_{ST}$ is significantly greater than zero.

Estimation of Regression Coefficient with Multiple Trait Model

In the multiple trait analysis, the regression of the phenotype on the MV is not estimated directly in the analysis, but can be estimated by $\hat{\sigma}_{p_{ym}}/\hat{\sigma}^2_{p_m}$. If the residual covariance was estimated, we will refer to it as $\hat{b}_{MT_e} = \hat{\sigma}_{p_{ym}}/\hat{\sigma}^2_{p_m}$. In the event that the analysis is for MAS and the residual covariance was set to zero, we will refer to it as $\hat{b}_{MT_0} = \hat{\sigma}_{p_{ym}}/\hat{\sigma}^2_{p_m} = \hat{\sigma}_{g_{ym}}/\hat{\sigma}^2_{p_m}$ as was used at http://www.beefcrc.com.au/Aus-Beef-DNA-results (accessed 4/15/09). In either case, it seems useful to compare these estimators with the direct estimator from the single trait model.

The approximate standard errors of $\hat{b}_{MT_e}$ and $\hat{b}_{MT_e}$ were computed as

$$se[\hat{b}_{MT_e}] \cong \left(\frac{\hat{\sigma}_{p_{ym}}}{\hat{\sigma}^2_{p_m}}\right)\left(\frac{Var(\hat{\sigma}_{p_{ym}})}{\hat{\sigma}_{p_{ym}}} + \frac{Var(\hat{\sigma}^2_{p_m})}{\hat{\sigma}^2_{p_m}} - 2\frac{Cov(\hat{\sigma}_{p_{ym}},\hat{\sigma}^2_{p_m})}{\hat{\sigma}_{p_{ym}}\,\hat{\sigma}^2_{p_m}}\right)^{1/2}$$

$$se[\hat{b}_{MT_0}] \cong \left(\frac{\hat{\sigma}_{g_{ym}}}{\hat{\sigma}^2_{p_m}}\right)\left(\frac{Var(\hat{\sigma}_{g_{ym}})}{\hat{\sigma}_{g_{ym}}} + \frac{Var(\hat{\sigma}^2_{p_m})}{\hat{\sigma}^2_{p_m}} - 2\frac{Cov(\hat{\sigma}_{g_{ym}},\hat{\sigma}^2_{p_m})}{\hat{\sigma}_{g_{ym}}\,\hat{\sigma}^2_{p_m}}\right)^{1/2}$$

which can be confirmed using Appendix 1.

Materials and Methods

For each of three levels of $h^2_{g_y}$ (0.1, 0.3, and 0.5) and four levels of R_g^2 (0.04, 0.16, 0.36, and 0.64), 500 independent replicates were simulated. Each replicate consisted of 100 unrelated sires with 10 offspring each from unrelated dams. Each of the progeny was randomly assigned to one of 20 contemporary groups. The phenotypes for the replicates with only additive effects were generated as the sum of a contemporary group effect, a sire effect, an additive genetic component of the residual, and an environmental component of the residual. The sire effects for the phenotypes and MV were generated as bivariate normal random variables each with mean zero, variance of $0.25 \times h^2_{g_y}$, and correlation equal to the genetic correlation ($r_g = \sqrt{R_g^2}$). The genetic component of the residuals was generated in the same way except with a variance of $.75 \times h^2_{g_y}$ and correlation equal to $\sqrt{R_g^2}$. The environmental component of the phenotype's residual was generated as a normal random variable with mean zero and variance of $1 - h^2_{g_y}$. The residual was then the sum of the genetic and environmental components. The resulting observed trait phenotype has phenotypic variance of 1. The MV were the sum of the sire effect on the MV (with variance = $0.25 \times h^2_{g_y}$) and the genetic component of the residuals for the MV (with a variance of $.75 \times h^2_{g_y}$). Thus, the MV had a variance equal to the observed trait heritability ($h^2_{g_y}$) and did

not include an error effect ($h^2_{g_m} = h^2_{t_m} = 1$). Because all the estimators were translation invariant, the contemporary group effects were all set to zero. Therefore, $\sigma_{g_{ym}} = h^2_{g_y} r_g$ and the additive genetic variance accounted for by **m**, was

$$\sigma^2_{g_y} - \sigma^2_{g_y|g_m} = \frac{(\sigma_{g_{ym}})^2}{\sigma^2_{g_m}} = \frac{(h^2_{g_y} r_g)^2}{h^2_{g_y}} = h^2_{g_y} r_g{}^2$$

and the regression of **y** on **m** is

$$b = \frac{\sigma_{p_{ym}}}{\sigma^2_{p_m}} = \frac{\sigma_{g_{ym}}}{\sigma^2_{g_m}} = \frac{h^2_{g_y} r_g}{h^2_{g_y}} = r_g.$$

The last two expressions are specific to the way in which these data were simulated; they are not true in general.

For each of the same twelve combinations of $h^2_{g_y}$ and $R_g{}^2$, an additional 500 replicates with non-additive genetic effects were generated in such a way that $h^2_{g_m}$ was equal to 0.8. An additional component representing non-additive genetic effects was simulated as a bivariate normal random variable. To achieve $h^2_{g_m} = 0.8$, the values of $\sigma^2_{na_m}$ and $\sigma^2_{na_y}$ were set to 0.25 x $h^2_{g_y}$, with the correlation between the non-additive contributions to **y** and **m** equal to r_g. In these replicates, the environmental component of the phenotype's residual was generated as a normal random variable with mean zero and variance of $1 - h^2_{g_y} - 0.25\, h^2_{g_y}$, so that $\sigma^2_{p_y}$ was still equal to 1. The broad sense heritability of MV, $h^2_{t_m}$ was equal to 1 in all replicates. Therefore, $\sigma_{na_{ym}} = 0.25\, h^2_{g_y} r_g$ and the non-additive genetic variance accounted for by **m**, was

$$\sigma^2_{na_y} - \sigma^2_{na_y|na_m} = \frac{(\sigma_{na_{ym}})^2}{\sigma^2_{na_m}} = \frac{(0.25\, h^2_{g_y} r_g)^2}{0.25\, h^2_{g_y}} = 0.25\, h^2_{g_y} r_g{}^2.$$

Consequently, the non-additive genetic variation accounted for in these replicates was 25% of the additive genetic variation accounted for in them. The regression of **y** on **m** is

$$b = \frac{\sigma_{p_{ym}}}{\sigma^2_{p_m}} = \frac{\sigma_{g_{ym}} + \sigma_{na_{ym}}}{\sigma^2_{g_m} + \sigma^2_{na_m}} = \frac{h^2_{g_y} r_g + 0.25\, h^2_{g_y} r_g}{h^2_{g_y} + 0.25\, h^2_{g_y}} = r_g.$$

The last two expressions are specific to the way in which these data were simulated; they are not true in general.

The single trait analyses were conducted using PROC Mixed of SAS[1] with an option that allows negative estimates of variances. Those estimates that fell within the parameter space were REML estimates and those that fell outside the parameter space are not REML. The two-trait analyses were conducted using ASReml[1]. Those parameters were within the parameter space with the exception of some numerical problems when the additive variance was on the boundary at zero in a preliminary analysis. The two-trait model was run for each replicate both with the residual correlations estimated and with them set to zero.

Results

Comparisons of the three alternative estimators for application in MAS are presented in Table 1. When simulated under an additive-only model, across the range

[1] Reference herein to any specific commercial products by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government, and shall not be used for advertising or product endorsement purposes.

of parameter sets considered, the mean of $\hat{R}_{g_{MT}}^2$ tended to be closer to the simulated values than $\hat{R}_{g_{RV}}^2$ and $\hat{R}_{g_{RPM}}^2$, although all three estimators performed reasonably for most parameter sets.

The most troublesome estimates were $\hat{R}_{g_{RV}}^2$ and $\hat{R}_{g_{RPM}}^2$ at $h^2_{g_y} = 0.1$. For $R_g^2 = 0.36$, two extreme outlier replicates for RV and one for RPM caused the means of 500 replicates to be $\hat{R}_{g_{RV}}^2 = 1.397$ and $\hat{R}_{g_{RPM}}^2 = 6.615$. With these extreme replicates removed, the means of the remaining replicates were 0.194 and 0.419 with standard deviations of 6.614 and 5.176, respectively. The means of $\hat{R}_{g_{MT}}^2$ with and without the residual constrained to zero, respectively, over the same replicates were 0.441 and 0.380 with standard deviations of 0.229 and 0.273. For $R_g^2 = 0.64$ ($h^2_{g_y} = 0.1$), outlier replicates also caused unreasonable means and huge standard deviations of estimates for both $\hat{R}_{g_{RV}}^2$ and $\hat{R}_{g_{RPM}}^2$. These outliers were caused by estimates of the sire variance (which is in the denominator of these estimators) that were very close to zero. Estimates of sire variance close to zero were uniformly distributed across the levels of R_g^2 and were primarily due to low heritability. It was a matter of chance that out of 2,000 replicates with $h^2_{g_y} = 0.1$, the most extreme (closest to zero) were at $R_g^2 = 0.36$ and next most extreme were at $R_g^2 = 0.64$.

However, $\hat{R}_{g_{MT}}^2$ tended to be biased up when the simulated value of R_g^2 was close to zero, relative to the mean standard error. This was particularly noticeable at low heritability, whether non-additive effects were simulated or not and whether the residual covariance was estimated or set to zero. At $h^2_{g_y} = 0.1$ and $R_g^2 = 0.64$, $\hat{R}_{g_{MT}}^2$ was seriously underestimated (regardless of whether non-additive effects were simulated (0.505) or not (0.536), but only when the residual covariance was estimated.

When non-additive genetic effects were simulated, the mean of $\hat{R}_{g_{RPM}}^2$ was considerably higher than the other estimators, especially for $h^2_{g_y} \geq 0.3$. This was expected because the derivation of this estimator is highly dependent on the assumption, implicit in the definition of an MBV, that it is strictly additive.

Excluding the combinations with $h^2_{g_y} = 0.1$ (for which the means were too erratic and the standard deviations far too high to draw any conclusions), $\hat{R}_{g_{RV}}^2$ showed no signs of bias, even when non-additive genetic effects were included in the simulation.

The root mean squared errors (**RMSE**) of $\hat{R}_{g_{MT}}^2$ were almost uniformly smaller than $\hat{R}_{g_{RV}}^2$ and $\hat{R}_{g_{RPM}}^2$; the only two notable exceptions were at $R_g^2 = 0.04$ with the residual covariance estimated and only $\hat{R}_{g_{RPM}}^2$ performed better than $\hat{R}_{g_{MT}}^2$. At $h^2_{g_y} = 0.1$, RMSE of $\hat{R}_{g_{MT}}^2$ were higher than desired, but reasonable, whereas RMSE of $\hat{R}_{g_{RV}}^2$ and $\hat{R}_{g_{RPM}}^2$ suggested those estimators were useless.

The $\hat{R}_{g_{RV}}^2$ estimator can produce negative estimates and both $\hat{R}_{g_{RV}}^2$ and $\hat{R}_{g_{RPM}}^2$ can produce estimates > 1 of the proportion of additive variance explained. Examples of each of these types of estimates outside the parameter space occurred.

The $\hat{R}_{g_{RPM}}^2$ estimator appears mathematically constrained to be non-negative, yet it's mean for several parameter combinations were negative. This occurred due to negative estimates of sire variance.

The approximate standard errors of $\hat{R}_{g_{MT}}^2$ that were computed as described above were generally very close to the standard deviations of the estimates. They certainly are close enough to serve as guides as to whether a data set is sufficient to estimate the proportion of variation reasonably well. The few exceptions in which the approximate standard error was considerably higher than the realized standard deviation and root mean squared error were those parameter sets for which the standard deviations of estimates were high, indicating that larger populations are required for reliable estimates.

When non-additive genetic effects were not simulated, the standard deviations and root mean squared errors of $\hat{R}_{g_{MT}}^2$ were uniformly smaller when the residual covariance was fixed at zero than when it was estimated. This was expected because there was one less parameter to be estimated and that parameter was set to the value used in the simulation. The mean estimates were similar whether $\sigma_{r_{ym}}$ was estimated or set to zero and, therefore, RMSE generally favored setting $\sigma_{r_{ym}} = 0$. This was especially pronounced at $R_g^2 = 0.04$, where RMSE was up to twice as high if the residual covariance was estimated as when it was fixed at zero.

When non-additive genetic effects were simulated, the standard deviations of $\hat{R}_{g_{MT}}^2$ were also uniformly smaller when the residual covariance was fixed at zero than when it was estimated. However, for all but one parameter combination ($h^2_{g_y} = 0.1$; $R_g^2 = 0.04$), the mean estimate was higher when the residual covariance was set to zero. For all but two parameter combinations, the mean estimate was closer to the simulated value when $\sigma_{r_{ym}}$ was estimated. The notable exception was ($h^2_{g_y} = 0.1$; $R_g^2 = 0.64$) in which case the estimate based on $\sigma_{r_{ym}} = 0$ was biased down considerably; however, the mean standard error of these estimates was quite high, suggesting that the population size was inadequate for this parameter combination. In all cases with $h^2_{g_y} \geq 0.3$ and $R_g^2 \geq 0.16$, setting $\sigma_{r_{ym}} = 0$ seemed to introduce an upward bias, in some cases quite large. Nonetheless, root mean squared error generally favored $\sigma_{r_{ym}} = 0$.

Table 2 presents comparisons of the three alternative estimators for application in MAM. In general, RMSE were considerably smaller for $\hat{R}_{p_{MT}}^2$, $\hat{R}_{p_{RV}}^2$, and $\hat{R}_{p_{RPM}}^2$ than for $\hat{R}_{g_{MT}}^2$, $\hat{R}_{g_{RV}}^2$, and $\hat{R}_{g_{RPM}}^2$. This was expected because the former estimators are not highly dependent on partitioning variation into additive genetic and residual components as are the latter. Furthermore, the denominator of each of these estimators contains the phenotypic variance, and therefore, is essentially assured of being substantially positive.

There was essentially no difference between the performance of $\hat{R}_{p_{MT}}^2$, $\hat{R}_{p_{RV}}^2$, and $\hat{R}_{p_{RPM}}^2$. All of them performed very well.

A comparison of the two methods of estimating the regression of phenotype on MV is presented in Table 3. There was essentially no difference between $\hat{b}_{\mathrm{MT}_e}$, $\hat{b}_{\mathrm{MT}_0}$, and

$\hat{b}_{ST}$. There was no indication of substantial bias and the RMSE were very similar. The sole minor exception was at ($h^2_{g_y}$ = 0.1; R_g^2 = 0.64) in which case $\hat{b}_{MT_e}$, and especially $\hat{b}_{MT_0}$, were underestimated slightly and had slightly greater standard deviations and RMSE than $\hat{b}_{ST}$.

The approximate standard errors of all three estimators were very similar to the standard deviations of the estimates, except for a tendency for $\hat{b}_{MT_e}$ and $\hat{b}_{MT_0}$ to be underestimated at $h^2_{g_y}$ = 0.1.

As a practical matter, any of the three estimators perform very well. The only concern with any of them is that hypothesis tests of $\hat{b}_{MT_e}$ and $\hat{b}_{MT_0}$ could be biased slightly toward significance for traits with low heritability.

Discussion

For some parameter combinations all of the estimators performed roughly similarly and acceptably well. However, for some parameter combinations (especially those involving low heritability and low proportion of genetic variation explained), the estimators based on RV and RPM produced some very erratic estimates. This problem is likely to become more pronounced in smaller data sets.

Furthermore, the RV estimators can produce negative estimates and the RV and RPM estimators can produce estimates > 1 of the proportion of variance explained. Provided REML is used to estimate the (co)variance parameters, the $\hat{R}_{g_{MT}}^2$ and $\hat{R}_{p_{MT}}^2$ estimators have the statistical properties of REML estimators, including the advantage of producing estimates within the parameter space. One of these desirable properties is that REML is a consistent estimator: as the sampling variance of the estimator diminishes (generally by increasing the sample size), the bias in the estimate also diminishes.

The data sets that are typically available for validation of DNA tests are far from ideal for partitioning additive from residual components of variation. Consequently, estimates of the proportion of additive genetic variation explained by MBV may have quite large standard errors. Therefore, these standard errors (or confidence intervals) should be computed and reported whenever the proportion of variation is reported. An additional advantage of the $\hat{R}_{g_{MT}}^2$ and $\hat{R}_{p_{MT}}^2$ estimators is that their standard errors can be readily computed. This is not feasible for the RV and RPM estimators because they are computed from different analyses of the same data.

It is often desirable to include contemporaries that have phenotypes, but no MV in an analysis to improve the estimation of contemporary group effects and the partitioning between additive and residual variation. But, because the RV and RPM estimators are fundamentally based on a single trait model that includes the MV as an independent variable, they inherently restrict the animals that can be included to those with both phenotype and MV. There is no such restriction in the multiple trait model; animals with either phenotype only or MV only can be handled just as easily as those with both phenotype and MV. Furthermore, the multiple trait approach can be readily extended to simultaneously handle the effects of multiple MV on an observed trait. It

can also handle the effects of an MV on multiple observed traits, accounting for the correlations among those traits.

Additionally, $\hat{R}_{g_{MT}}^2$ and $\hat{R}_{p_{MT}}^2$ have the intuitive appeal that they represent proportions of variation (similar to the common R^2 in statistics) and they are the squares of intuitively relevant correlations.

Considering all these factors and the general availability of multiple trait software that can compute standard errors of (co)variance parameters, it seems reasonable to recommend that estimation of proportions of variation be done with the $\hat{R}_{g_{MT}}^2$ and $\hat{R}_{p_{MT}}^2$ estimators. Because there seems to be little justification for using other estimators, the remainder of the paper assumes that the $\hat{R}_{g_{MT}}^2$ and $\hat{R}_{p_{MT}}^2$ estimators will be used.

Practical Considerations in Application

In computing $\hat{R}_{g_{MT}}^2$ and $\hat{R}_{p_{MT}}^2$ estimators for MAS, we must evaluate both cases in which the MV is assumed to be completely additive (our definition of a true MBV) and cases in which the MV presented as an MBV includes non-additive components. For tests intended for use in MAS, it could be useful to evaluate $\hat{R}_{p_{MT}}^2$ in addition to $\hat{R}_{g_{MT}}^2$, because $\hat{R}_{p_{MT}}^2$ can be estimated more accurately and $\hat{R}_{p_{MT}}^2 / h^2_{g_y}$ provides an estimate of the upper limit of $R_{g_{MT}}^2$. However, $\hat{R}_{g_{MT}}^2$, is not relevant for evaluating MGV that aim to account for non-additive genetics and breed effects for application to MAM.

For applications in MAS, there are advantages to setting the residual correlation to zero, but only if it is certain that the MBV are computed using a strictly additive model. Otherwise, the residual correlation should be estimated. For applications in MAM, the residual correlation should always be estimated.

If $\hat{h}^2_{g_m}$ is very close to 1, then $\hat{\sigma}^2_{r_m}$ must be very small, and consequently, $|\hat{\sigma}_{r_{ym}}|$ must also be small. Therefore, it seems unlikely that whether $\hat{\sigma}_{r_{ym}}$ is estimated or not could have much effect on $\hat{r}_g$ under this condition, even though the residual correlation could vary considerably. However, the accuracy of estimating $\hat{r}_g$ was affected by whether $\hat{\sigma}_{r_{ym}}$ was estimated or not. Therefore, it seems likely that this difference was due primarily to those (less frequent) replicates in which $h^2_{g_m}$ was underestimated considerably and that, in those replicates, the effect of estimating the residual correlation or not may be considerably larger than is reflected in Table 1. This should be explored more fully.

If $\hat{h}^2_{g_m}$ is significantly less than 1, then its estimated value should be used for the multiple trait analysis. It is not completely clear what the best approach is if $\hat{h}^2_{g_m}$ is substantially, but not significantly less than 1. The default position would seem to be that $\hat{\sigma}^2_{r_m}$ should be estimated unless it is close enough to the boundary value of zero ($\hat{h}^2_{g_m} = 1$) to cause numerical problems. However, it could be argued that the natural value of $h^2_{g_m}$ is just slightly less than one, and therefore, $\hat{\sigma}^2_{r_m}$ should be set to a value slightly greater than zero unless $\hat{h}^2_{g_m}$ is significantly less than one.

Therefore, it is reasonable to ask not only whether the residual covariance should be estimated or set equal to zero, but also whether $\hat{\sigma}^2_{r_m}$ should be estimated or set to a small positive value (regardless of whether the estimate hits a parameter space boundary or not). It appears that the latter question may be more important than the first, but this requires further investigation. To some extent, the answers to both of these questions depend on how much information the DNA testing companies are willing to provide regarding how their MV are constructed.

It is possible that the estimate of the genetic correlation could be negative, in which case the squared correlation would be exactly the same as if the estimate had been the absolute value of the observed correlation. Although this is theoretically correct, the MV are claimed to be in the same direction as the observed trait (allowing one-tailed tests to be applied). Therefore, if the estimated genetic correlation is negative, the estimate of the proportion of genetic variation explained should be considered to be 0 or undefined and the MV should not be included in NCE. We recommend that such results should be reported as * and footnoted to indicate that the estimate is undefined because the genetic correlation was in the direction opposite to the claim.

However, if a trait for which no claim exists is included in the analysis, then it may be perfectly appropriate to square a negative genetic correlation, just as it would be appropriate, in that case, to apply a two-tailed hypothesis test. An example of such a circumstance could be an MBV for marbling being analyzed along with phenotypes for marbling and growth with the DNA testing company's claim being only for marbling. Then, the proportion of variation for growth would be the square of the genetic correlation, whether negative or positive, but for marbling, the proportion of variation would be undefined if that genetic correlation was negative.

The standard error of $\hat{R}_{g_{MT}}{}^2$ is at least partly a function of how well the additive genetic variances of both the observed trait and the MBV can be partitioned from the residual variances. This is likely to be highly dependent on the pedigree structure. It seems likely that multigeneration pedigrees analyzed with the full relationship matrix may be considerably more important for the purpose of estimating these (co)variances than they have been for the purpose of validating that a test "works". Specifically, it may be useful to have phenotypes and MBV on dam-offspring pairs, so that the dam side of the pedigree can contribute (in addition to the sire side) to partitioning the additive genetic component from the residual. For those traits for which phenotypes are plentiful (those included in NCE) it could be very efficient to run the DNA tests (and include the MBV) on large numbers of sires that have many progeny with phenotypes.

Eventually, MBV for many seedstock animals (primarily without phenotypes for the target trait) should become available (this is a prerequisite to use the DNA tests in NCE). When this data becomes available, it may be helpful to include it in the analysis for the (co)variance estimation in order to better partition the additive from residual variance of MBV and to better estimate the residual covariance between MBV and observed traits. When this option becomes available, it seems likely that the advantages of making fewer assumptions regarding the MBV will outweigh the advantages of estimating fewer parameters that were discussed above.

In general, relatively few fixed effects need be fit in the model for the MV in the multiple trait approach; in some cases only an overall mean may be sufficient. There

seems to be no reason to fit the contemporary group effects that are appropriate for the observed traits.

For application in MAS, in general, it seems most appropriate that breed effects should be included in the statistical model for both the observed and MBV traits. Thus the (co)variance parameters estimated would be free of breed effects and, therefore, appropriate for within-breed selection. However, for tests that are optimized for selection in *Bos indicus* × *B. taurus* composites, it may be appropriate for the DNA tests to account for the breed effects, in which case, the (co)variance parameters should include them (the breed effects should be dropped from the model). This argument could be extended to include selection in *B. taurus* composites. It is not clear whether there are circumstances in which it would be appropriate to include breed effects in the model for observed traits, but not the MBV traits, or *vice versa*. It is an issue that requires study.

The current NBCEC position is that (co)variance estimates for application in MAS should be obtained and reported with models that account for breed effects for both the observed and MBV traits and that knowledge of breed composition will remain an important attribute of populations that are appropriate for (co)variance parameter estimation. It would be reasonable for DNA testing companies to request the estimation and reporting of (co)variance parameters from other models as well, provided that footnotes explaining the different interpretation of those results are included in the report. Obviously, if companies wish to exercise this option, they need to specify that at the time the validation is requested.

For application in MAM, it is less clear whether breed effects should be fit in the models for phenotypes and MGV. One of the opportunities in MAM is to use the markers' ability to estimate breed composition to enhance the prediction of total genetic merit. Omitting breed effects seems appropriate in analyses for the validation of tests intended to be used for MAM in mixed breed populations in which breed composition is unknown (a common situation in feedlot applications). However, if the intended application of DNA tests is MAM within populations that are uniform in breed composition, it will be more useful to evaluate the tests with models that include breed effects. Evaluating tests with both models that include and do not include breed effects may be useful in determining the extent to which the tests' predictive ability is derived from the estimation of breed composition.

The single trait models were fit as sire models because that is the model that has typically been used in NBCEC's independent validations of DNA tests and those analyses have typically been conducted using software that does not easily incorporate the numerator relationship matrix. The multiple trait model was fit as an animal model because that is how analyses will likely be conducted in practice and it would be the most reasonable alternative if populations with more complicated pedigrees were encountered. For the simulated pedigree structure, the sire and animal models are equivalent, with one exception: in the animal model, the upper bound on narrow sense heritability is one, but with the sire model, the upper bound on heritability is four. This is a minor difference in computation of the estimators that is distinct from the fundamental properties of the estimators themselves, but it seems unlikely to have changed the results noticeably. Keeping the heritability within its natural parameter space is an advantage of the animal model.

Replicates in which $\hat{\sigma}^2_{g_y}$ was at or near zero (the boundary of the parameter space) gave very poor results for estimating R_g^2 with any of the estimators. Obviously, it is very difficult to estimate a proportion of something (additive genetic variance) that is very close to zero. Had the SAS option to allow negative variances been turned off, many of the estimates of sire variance would have been zero (which would have made $\hat{R}_{g_{RV}}^2$ and $\hat{R}_{g_{RPM}}^2$ undefined) or very close to zero (which makes $\hat{R}_{g_{RV}}^2$ and $\hat{R}_{g_{RPM}}^2$ unreasonably large).

This is not a problem that is unique to unusual replicates of simulated data. In real data, $\hat{\sigma}^2_{g_y}$ could be near zero either due to sampling variation in high quality data with reasonable heritability or because of data quality problems that cause the effective heritability of a data set to be low for a trait that typically is moderate or high in heritability. In either case, the data set will have limited value for estimating the proportion of additive variation and, if practical, should be replaced with another data set with higher estimated heritability. Caution should similarly be exercised if the heritability of MBV is lower than expected.

In third party independent processes such as described by (Van Eenennaam *et al*., 2009), it is critical that data quality checks are conducted prior to estimation of R_g^2. If the sequence is reversed, the data quality checks are typically done only when the estimate of R_g^2 is disappointing; this obviously introduces bias into the estimates. Moreover, it unnecessarily creates situations in which decisions about whether or not to remove questionable data sets can not be made as objectively as would have otherwise been possible. To be implemented most effectively, the company presenting a test for validation would review summary statistics on data quality (of both observed traits and MBV) and agree to include the data set prior to the commencement of any estimation of R_g^2 or other analyses of association between the observed traits and MBV.

The problem with $\hat{\sigma}^2_{g_y}$ being nearly zero obviously occurs much more frequently when the heritability is low and/or the standard error is too large because of population size and structure. Considerably larger populations will be required for traits with low heritability.

For application in MAM, $\hat{\sigma}^2_{p_y}$ hitting the boundary at zero (the denominator of $\hat{R}_{p_{MT}}^2$ approaching zero) is unlikely to be experienced and cause numerical problems. However, issues with data quality will be just as important in MAM, but different diagnostic statistics will be required, depending on the nature of the data set.

The REML estimates of R_g^2 were biased up when heritability of the observed trait and the genetic correlation were low. The obvious solution to this problem seems to be to add more data if standard errors are too high. However, it is likely that this solution will come in the form of more data sets of similar size rather than larger data sets. If all the data sets could be pooled for a joint analysis, the resulting standard errors would be much lower and it seems likely that the bias would decrease substantially accordingly. However, the practical situation is more likely to be that the data sets cannot be pooled and that we will have separate analyses, each with potentially large standard errors and bias. In this case, a properly conducted metaanalyses should account for this property of REML and produce estimates comparable to what a joint analysis would have yielded. But, a weighted average of the estimates will not have this property and

"eyeball estimates" attempting to combine various studies mentally will be even worse. Therefore, it will be important to use appropriate metaanalysis techniques that account for this property.

In conducting its process for independent (co)variance estimation/validation, NBCEC should consider setting a minimum standard error on estimates of R_g^2 and R_p^2. These could be on a per population basis and/or for the pooled and/or metaanalysis results.

In the context of MAS, the estimates of the regression coefficients are probably more important in the short term than they will be when the incorporation of DNA testing into the NCE system becomes complete. The reason they are important in the short term is that they serve essentially as a scaling factor for translating between the units of MBV and the units of EPD. They typically are reported in identical units, but this can be misleading. First, MBV are predictors of breeding value, while EPD predict half of breeding value. But more importantly, if *b* is substantially less than one, the MBV overstate the effect of the test, in terms of how users perceive the MBV relative to EPD. Low values of *b* are not good or bad, they just change how the MBV should be evaluated relative to EPD.

Once MBV are incorporated into NCE and are (optimistically) no longer evaluated by users directly alongside EPD, the issue of how MBV are scaled should become inconsequential. In the multiple trait model, the output of importance is the EPD of the observed trait, presented in its current units. However, its accuracy should be improved due to the correlations with MBV (MacNeil *et al*., 2009).

The regressions of observed phenotypes on MBV for tests that have gone through the NBCEC validation process have ranged from about 0.2 to 1. There are several reasons for this: the discovery process favors SNP whose effects are overestimated in the discovery data, it is likely that some of the SNP included in the MBV are false positives (have no effect on the observed trait) and those SNP that are actually predicting genetic effects on the observed trait are unlikely to be the causative SNP (there is an imperfect correlation between the SNP effect and the part of the genetic effect on the observed trait that they are predicting). It makes no practical difference what the cause of the regression being different from one is.

In past NBCEC validations, the primary criteria for evaluating MBV has been a test of the null hypothesis that $b = 0$ (i.e., did the test perform significantly better than a test with no value?). We envision that the process will evolve into one primarily focused on the estimation of the variances and covariances required to integrate MBV into NCE. As shown here, the proportion of additive genetic variation accounted for by the MBV is a fundamental byproduct of these (co)variances and we believe it is a better metric with which to evaluate DNA tests than the significance level of $b \neq 0$.

In the context of MAM, the issue of scaling and the regression of phenotype on MGV is likely to be more important and persist longer because the fundamental purpose of the tests is to predict phenotypic differences rather than ranking animals and because it seems unlikely that there will be an equivalent to NCE to automatically transform MGV to a practically useful scale.

It has been suggested to test the null hypothesis that $b = 1$ (in this case, a lack of significant difference from one is desired). However, there are some problems with this approach: the scale of phenotypes is not uniform across environments and production

systems, even within the U.S. beef industry. The NCE system expresses EPD on a scale that is essentially averaged over the seedstock individuals within a breed. However, it should not surprise us if the phenotypes in any one particular population were on a scale significantly different from the NCE scale (see Kuehn *et al*., 2009 for examples in which the regression of progeny phenotype on EPD are different from one) or on the scale of any particular MGV intended for use across a broad range of environments and production systems.

Therefore, it might be more useful to test the null hypothesis that $0.8 \leq b \leq 1.25$. This would allow for some range in the scale of the MGV. However, if the scale was too far off for certain production conditions, it would indicate that the MGV should be rescaled (at the minimum), or perhaps reestimated and that different versions of MGV (perhaps derived from a common set of SNP) should be used for those different production conditions.

Assuming the multiple trait approach is used for estimation of R_g^2 and/or R_p^2, it will be more convenient to estimate b with $\hat{b}_{MT_0}$ or $\hat{b}_{MT_e}$ and, in most cases, this should be perfectly acceptable. However, if important decisions are to be made based on hypothesis tests and the significance values are close to the threshold, it would be preferable to use $\hat{b}_{ST}$.

It is certainly not necessary to use REML to estimate the proposed statistics. Gibbs sampling could also be used to obtain Bayesian estimates, including the full posterior distribution. This approach should handle the boundary conditions better and would not require approximation of the standard error. It should be computationally feasible for the size of data sets that are likely to be available.

In order for an estimate of proportion of variation to be useful, it is critical that the MV not be derived from the same (or a very closely related population) as is used to estimate proportion of variation. Otherwise, the estimate of proportion of variation will be biased up, perhaps very seriously. Finding appropriate populations can be a considerable challenge for traits for which phenotypes are quite limited (e.g., residual feed intake). A practical guideline is that the average relationship between the animals in the discovery data and those in the data from which the proportion of variation will be estimated should not exceed the average relationship between the discovery population and the target population for application of the test.

Some rare alleles with large, favorable effects may have substantial economic value while accounting for little current genetic variation. Such tests are valuable because they have the potential, given sufficient selection emphasis and time, to account for considerable genetic variation in the future. Therefore, in the future, it will be beneficial to develop methods to estimate potential future genetic variation accounted for by a test. However, this seems unfeasible for the current generation of DNA tests for which the results are presented only as MV, without the individual genotypes. In this situation, it is impossible to compute allele frequencies and effects of individual SNP, and this is the essence of the information required to estimate potential future genetic variation. Therefore, for the immediate future, it seems most practical to focus on current genetic variation, which will be challenging enough by itself. When that challenge has been substantially met, we can shift the focus to finding feasible approaches to estimating potential future variation.

Although proportion of additive genetic variation is a useful metric for evaluating DNA tests intended for use in MAS, the form that seedstock breeders will find easier to interpret is how much does the test increase the accuracy of EPD on an animal that already has a certain base level of accuracy (the most relevant is probably the typical accuracy for the trait of a yearling animal in the breeder's herd). A table illustrating this increase in accuracy for a specific example can be found at http://www.bifconference.com/bif2008/ppt/Stephen%20Kachman_GP.pdf (accessed 4/15/09). A formula or table that is fairly general would be a useful contribution to the field.

Conclusions

We consider the reporting of percentage of genetic variation accounted for by DNA tests to be an integral part of the validation process in the short term. Furthermore, it is an important step in the longer term transition away from validating whether a test "works" or not toward the increasingly relevant process of estimating the (co)variance parameters. This estimation will be required to incorporate DNA testing into the national cattle evaluation system, and thus, for the beef industry to utilize this technology much more effectively and extensively than it is currently being utilized.

The proportion of variation accounted for by DNA tests should be a very useful tool for cattle producers to use in determining the value of DNA tests in their breeding programs and production systems.

The NBCEC statistical team considers the squared genetic correlation between the observed trait and the MBV from the multiple trait model ($\hat{R}_{g_{MT}}^2$) to be the best estimator of proportion of genetic variation accounted for by a DNA test (in the form of an MBV). Therefore, until further notice, NBCEC will compute proportion of genetic variation with this method in all future validation analyses and will require that it be computed in this way in third party analyses that are to be used in NBCEC validation. Furthermore, we recommend that henceforth this estimator be referred to simply as $\hat{R}_g^2$. Similarly, $\hat{R}_{p_{MT}}^2$ is recommended as the best estimator of the proportion of phenotypic variance accounted for by an MGV for use in MAM and we recommend that it be referred to simply as $\hat{R}_p^2$.

References

Kachman, S. D. 2008. Parameters needed to add genomics to genetic prediction. Proc. of the 9th Genetic Prediction Workshop, Beef Improvement Federation, Kansas City, MO, Dec. 8-10, pp 92-98.

Kuehn, L. A., L. D. Van Vleck, R. M. Thallman and L. V. Cundiff. 2009. Across-breed EPD tables for the year 2009 adjusted to breed differences for birth year of 2007. Proc. of the Beef Improvement Federation, Sacramento, CA, Apr. 30-May 3 (in press).

Lynch, M., and B. Walsh. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer Associates. Sunderland, MA.

MacNeil, M. D., J. D. Nkrumah, and S. L. Northcutt. 2009. Genetic evaluation of Angus cattle for carcass marbling using ultrasound and genomic indicators. J. Anim. Sci. (submitted).

Moser, D. W. 2008. Marketing the covariances: enhancing the utilization of genomic tools in beef cattle improvement. Proc. of the 9th Genetic Prediction Workshop, Beef Improvement Federation, Kansas City, MO, Dec. 8-10, pp 99-100.

Oehlert, G.W. 1992. A note on the delta method. The American Statistician. 46:27-29.

Van Eenennaam, A.L., J. Li, R.M. Thallman, R.L. Quaas, M.E. Dikeman, C. Gill, D.E. Franke, and M.G. Thomas. 2007. Validation of commercial DNA tests for quantitative beef quality traits. J. Anim. Sci. 85:891-900.

Van Eenennaam, A. L., R. M. Thallman, R. L. Quaas, K. Hanford, and E. J Pollak. 2009. Validation and Estimation of Additive Genetic Variation Associated with DNA Tests for Quantitative Beef Cattle Traits. Proceedings of the 18th Conference of the Association for the Advancement of Animal Breeding and Genetics, Queensland, Australia, September 28 – October 1. (in press).

Appendix 1

Standard errors of functions of variance components can be obtained using the delta method (e.g., Lynch and Walsh, 1998, Appendix 1). Let $f(\hat{\theta}_1, \hat{\theta}_2,\ldots, \hat{\theta}_k)$ be a function of k variance components $\theta_1, \theta_2,\ldots, \theta_k$. An approximate variance of $f(\hat{\theta}_1, \hat{\theta}_2,\ldots, \hat{\theta}_k)$ can be found using

$$\text{Var}(f(\hat{\theta}_1, \hat{\theta}_2,\ldots, \hat{\theta}_k)) = \sum_{i=1}^{k}\left(\frac{\partial f}{\partial \theta_i}\right)^2 \text{Var}(\hat{\theta}_i) + 2\sum_{i=2}^{k}\sum_{j=1}^{i-1}\left(\frac{\partial f}{\partial \theta_i}\right)\left(\frac{\partial f}{\partial \theta_j}\right)\text{Cov}(\hat{\theta}_i,\hat{\theta}_j)$$

An approximate standard error can then be found by taking the square root of the approximate variance. Formulas for some common functions can be found in Table A1.

Table A1. Approximate variance formulas using the delta method for some common functions

Function	Approximate Variance
$\hat{\theta}^2$	$4\,\theta^2\,\text{Var}(\hat{\theta})$
$\frac{\hat{\theta}_1}{\hat{\theta}_2}$	$\left(\frac{\theta_1}{\theta_2}\right)^2\left(\frac{\text{Var}(\hat{\theta}_1)}{\theta_1^2} + \frac{\text{Var}(\hat{\theta}_2)}{\theta_2^2} - 2\,\frac{\text{Cov}(\hat{\theta}_1,\hat{\theta}_2)}{\theta_1\theta_2}\right)$
$\frac{\hat{\theta}_{12}}{\sqrt{\hat{\theta}_1\hat{\theta}_2}}$	$\left(\frac{\hat{\theta}_{12}}{\sqrt{\theta_1\theta_2}}\right)^2\left(\frac{\text{Var}(\hat{\theta}_{12})}{\theta_{12}^2} + \frac{\text{Var}(\hat{\theta}_1)}{4\theta_1^2} + \frac{\text{Var}(\hat{\theta}_2)}{4\theta_2^2} - \frac{\text{Cov}(\hat{\theta}_{12},\hat{\theta}_1)}{\theta_{12}\theta_1} - \frac{\text{Cov}(\hat{\theta}_{12},\hat{\theta}_2)}{\theta_{12}\theta_2} - \frac{\text{Cov}(\hat{\theta}_1,\hat{\theta}_2)}{2\theta_1\theta_2}\right)$

Table 1. Proportion of Additive Variance Explained by MBV

Model:		Two Trait				Two Trait				Full & Reduced ST		Full Single Trait	
Res. Cov:		Estimated				Constrained to Zero				N/A		N/A	
Estimator:		Genetic Correlation Squared $\hat{R}_{g_{MT}}^2$				Genetic Correlation Squared $\hat{R}_{g_{MT}}^2$				Reduction in Variance $\hat{R}_{g_{RV}}^2$		Regression on MBV $\hat{R}_{g_{RPM}}^2$	
Simulation Parameters h^2_{gy}	R_g^2	Mean Estimate ± Standard Error of Mean	Root Mean Sq Error	Mean Std Error of Est	Std Dev of Est	Mean Estimate ± Standard Error of Mean	Root Mean Sq Error	Mean Std Error of Est	Std Dev of Est	Mean Estimate ± Standard Error of Mean	Root Mean Sq Error	Mean Estimate ± Standard Error of Mean	Root Mean Sq Error
Data Simulated from Additive Model Only													
0.1	0.04	0.113 ± 0.008	0.184	0.262	0.169	0.082 ± 0.005	0.114	0.174	0.106	0.018 ± 0.019	0.427	0.048 ± 0.018	0.404
0.1	0.16	0.205 ± 0.010	0.228	0.256	0.224	0.230 ± 0.009	0.206	0.259	0.193	0.171 ± 0.084	1.886	0.214 ± 0.073	1.644
0.1	0.36	0.380 ± 0.012	0.274	0.289	0.273	0.441 ± 0.010	0.243	0.264	0.229	1.397 ± 1.102[a]	6.616	6.615 ± 6.201[b]	138.810
0.1	0.64	0.536 ± 0.012	0.290	0.196	0.271	0.641 ± 0.010	0.214	0.220	0.214	0.285 ± 0.551[c]	9.885	-0.234 ± 0.587	17.029
0.3	0.04	0.061 ± 0.003	0.072	0.051	0.069	0.050 ± 0.001	0.035	0.033	0.033	0.036 ± 0.004	0.079	0.049 ± 0.001	0.034
0.3	0.16	0.169 ± 0.005	0.118	0.093	0.118	0.185 ± 0.003	0.081	0.076	0.077	0.152 ± 0.008	0.184	0.196 ± 0.014	0.324
0.3	0.36	0.352 ± 0.007	0.157	0.120	0.157	0.395 ± 0.005	0.127	0.117	0.123	0.351 ± 0.008	0.178	0.419 ± 0.008	0.196
0.3	0.64	0.636 ± 0.007	0.160	0.128	0.160	0.681 ± 0.006	0.146	0.132	0.140	0.661 ± 0.010	0.229	0.834 ± 0.052	1.184
0.5	0.04	0.049 ± 0.002	0.053	0.038	0.052	0.047 ± 0.001	0.025	0.024	0.024	0.036 ± 0.003	0.062	0.045 ± 0.001	0.025
0.5	0.16	0.156 ± 0.004	0.095	0.066	0.094	0.178 ± 0.003	0.060	0.053	0.058	0.156 ± 0.005	0.108	0.175 ± 0.003	0.072
0.5	0.36	0.351 ± 0.005	0.117	0.088	0.117	0.386 ± 0.003	0.078	0.079	0.074	0.355 ± 0.006	0.127	0.394 ± 0.006	0.129
0.5	0.64	0.629 ± 0.005	0.111	0.090	0.111	0.658 ± 0.004	0.092	0.086	0.090	0.630 ± 0.006	0.124	0.716 ± 0.014	0.313
Data Simulated from Additive & Non-Additive Model													
0.1	0.04	0.138 ± 0.008	0.209	0.340	0.184	0.116 ± 0.007	0.170	0.192	0.152	0.166 ± 0.059	1.324	-0.092 ± 0.129	2.879
0.1	0.16	0.249 ± 0.011	0.256	0.316	0.240	0.305 ± 0.009	0.245	0.251	0.198	-0.132 ± 0.159	3.556	-0.129 ± 0.242	5.424
0.1	0.36	0.379 ± 0.011	0.249	0.251	0.248	0.488 ± 0.008	0.227	0.234	0.188	0.317 ± 0.158	3.527	0.306 ± 0.261	5.840
0.1	0.64	0.505 ± 0.010	0.261	0.247	0.224	0.681 ± 0.007	0.167	0.199	0.162	0.627 ± 0.420	9.399	0.835 ± 1.017	22.752
0.3	0.04	0.073 ± 0.004	0.098	0.077	0.092	0.075 ± 0.002	0.063	0.047	0.053	0.037 ± 0.004	0.096	0.078 ± 0.003	0.071
0.3	0.16	0.200 ± 0.006	0.145	0.126	0.139	0.255 ± 0.004	0.136	0.092	0.097	0.167 ± 0.007	0.153	0.288 ± 0.006	0.185
0.3	0.36	0.377 ± 0.007	0.153	0.157	0.152	0.480 ± 0.005	0.166	0.117	0.115	0.345 ± 0.012	0.264	0.680 ± 0.025	0.654
0.3	0.64	0.650 ± 0.006	0.141	0.146	0.140	0.744 ± 0.005	0.151	0.113	0.110	0.651 ± 0.009	0.194	1.169 ± 0.028	0.824
0.5	0.04	0.060 ± 0.003	0.065	0.059	0.061	0.070 ± 0.001	0.044	0.032	0.033	0.036 ± 0.003	0.065	0.071 ± 0.002	0.048
0.5	0.16	0.176 ± 0.005	0.103	0.100	0.102	0.240 ± 0.003	0.103	0.064	0.064	0.154 ± 0.005	0.114	0.272 ± 0.004	0.146
0.5	0.36	0.375 ± 0.006	0.125	0.119	0.124	0.469 ± 0.004	0.136	0.080	0.082	0.359 ± 0.006	0.131	0.600 ± 0.009	0.307
0.5	0.64	0.637 ± 0.005	0.113	0.107	0.113	0.721 ± 0.003	0.107	0.070	0.070	0.623 ± 0.006	0.126	1.113 ± 0.016	0.595

[a] This cell included two replicates with extreme values. The mean of the remaining 498 replicates was 0.194 with a standard deviation of 6.614.

[b] This cell included one replicate with an extreme value. The mean of the remaining 499 replicates was 0. 419 with a standard deviation of 5.177.

[c] This cell included one replicate with an extreme value. The mean of the remaining 499 replicates was -0.049 with a standard deviation of 9.861.

Table 2. Proportion of Phenotypic Variance Explained by MGV

Model:			Two Trait				Full & Reduced ST		Full Single Trait	
Residual Covariance:			Estimated				N/A		N/A	
Estimator:			Genetic Correlation Squared $\hat{R}_{p_{MT}}^2$				Reduction in Variance $\hat{R}_{p_{RV}}^2$		Regression on MBV $\hat{R}_{p_{RPM}}^2$	
Simulation Parameters										
h^2_{gy}	R_g^2	R_p^2	Mean Estimate ± Standard Error of Mean	Root Mean Sq Error	Mean Std Error of Est	Std Dev of Est	Mean Estimate ± Standard Error of Mean	Root Mean Sq Error	Mean Estimate ± Standard Error of Mean	Root Mean Sq Error
Data Simulated from Additive Model Only										
0.1	0.04	0.004	0.0050 ± 0.0002	0.0043	0.0039	0.0042	0.0041 ± 0.0002	0.0043	0.0051 ± 0.0002	0.0045
0.1	0.16	0.016	0.0158 ± 0.0002	0.0078	0.0073	0.0078	0.0153 ± 0.0004	0.0081	0.0164 ± 0.0004	0.0080
0.1	0.36	0.036	0.0362 ± 0.0002	0.0149	0.0107	0.0149	0.0361 ± 0.0005	0.0121	0.0371 ± 0.0005	0.0121
0.1	0.64	0.064	0.0595 ± 0.0002	0.0165	0.0119	0.0158	0.0634 ± 0.0007	0.0152	0.0644 ± 0.0007	0.0153
0.3	0.04	0.012	0.0127 ± 0.0002	0.0070	0.0071	0.0070	0.0118 ± 0.0003	0.0072	0.0130 ± 0.0003	0.0074
0.3	0.16	0.048	0.0481 ± 0.0002	0.0136	0.0137	0.0136	0.0480 ± 0.0006	0.0142	0.0493 ± 0.0006	0.0141
0.3	0.36	0.108	0.1064 ± 0.0002	0.0193	0.0191	0.0192	0.1073 ± 0.0009	0.0199	0.1086 ± 0.0009	0.0196
0.3	0.64	0.192	0.1893 ± 0.0002	0.0238	0.0224	0.0237	0.1923 ± 0.0011	0.0243	0.1935 ± 0.0011	0.0244
0.5	0.04	0.020	0.0200 ± 0.0002	0.0092	0.0089	0.0092	0.0193 ± 0.0004	0.0097	0.0206 ± 0.0004	0.0089
0.5	0.16	0.080	0.0772 ± 0.0002	0.0184	0.0166	0.0182	0.0792 ± 0.0008	0.0182	0.0803 ± 0.0008	0.0176
0.5	0.36	0.180	0.1758 ± 0.0002	0.0249	0.0229	0.0245	0.1784 ± 0.0011	0.0240	0.1795 ± 0.0010	0.0228
0.5	0.64	0.320	0.3139 ± 0.0002	0.0265	0.0256	0.0258	0.3166 ± 0.0012	0.0274	0.3173 ± 0.0012	0.0263
Data Simulated from Additive & Non-Additive Model										
0.1	0.04	0.005	0.0059 ± 0.0002	0.0047	0.0044	0.0046	0.0049 ± 0.0002	0.0048	0.0060 ± 0.0002	0.0049
0.1	0.16	0.020	0.0212 ± 0.0004	0.0093	0.0087	0.0093	0.0205 ± 0.0004	0.0093	0.0216 ± 0.0004	0.0094
0.1	0.36	0.045	0.0455 ± 0.0006	0.0131	0.0125	0.0131	0.0457 ± 0.0006	0.0133	0.0466 ± 0.0006	0.0133
0.1	0.64	0.080	0.0764 ± 0.0008	0.0176	0.0149	0.0172	0.0788 ± 0.0008	0.0171	0.0798 ± 0.0008	0.0171
0.3	0.04	0.015	0.0161 ± 0.0004	0.0083	0.0081	0.0082	0.0150 ± 0.0004	0.0085	0.0161 ± 0.0004	0.0081
0.3	0.16	0.060	0.0613 ± 0.0007	0.0158	0.0154	0.0157	0.0603 ± 0.0007	0.0159	0.0611 ± 0.0007	0.0156
0.3	0.36	0.135	0.1344 ± 0.0010	0.0214	0.0210	0.0214	0.1337 ± 0.0010	0.0217	0.1346 ± 0.0010	0.0213
0.3	0.64	0.240	0.2393 ± 0.0010	0.0230	0.0243	0.0230	0.2396 ± 0.0011	0.0235	0.2400 ± 0.0010	0.0231
0.5	0.04	0.025	0.0258 ± 0.0005	0.0108	0.0106	0.0107	0.0246 ± 0.0005	0.0109	0.0259 ± 0.0005	0.0103
0.5	0.16	0.100	0.0999 ± 0.0009	0.0195	0.0196	0.0195	0.0992 ± 0.0009	0.0199	0.1003 ± 0.0008	0.0188
0.5	0.36	0.225	0.2249 ± 0.0011	0.0249	0.0252	0.0249	0.2241 ± 0.0011	0.0251	0.2245 ± 0.0011	0.0248
0.5	0.64	0.400	0.3988 ± 0.0011	0.0257	0.0258	0.0256	0.3983 ± 0.0012	0.0262	0.3999 ± 0.0011	0.0255

Table 3. Regression of Observed Trait Phenotypes on MBV

Model:			Two Trait				Two Trait				Full Single Trait			
Res. Cov:			Estimated				Constrained to Zero				N/A			
Estimator:			Genetic (Co)variance Parameter Est. $\hat{b}_{MT_e}$				Genetic (Co)variance Parameter Est. $\hat{b}_{MT_0}$				Estimated Regression on MBV $\hat{b}_{ST}$			
Simulation Parameters h^2_{gy}	R_g^2	b	Mean Estimate ± Standard Error of Mean	Root Mean Sq Error	Mean Std Error of Est	Std Dev of Est	Mean Estimate ± Standard Error of Mean	Root Mean Sq Error	Mean Std Error of Est	Std Dev of Est	Mean Estimate ± Standard Error of Mean	Root Mean Sq Error	Mean Std Error of Est	Std Dev of Est
Data Simulated from Additive Model Only														
0.1	0.04	0.2	0.200 ± 0.004	0.100	0.098	0.100	0.199 ± 0.005	0.102	0.097	0.102	0.203 ± 0.005	0.102	0.103	0.102
0.1	0.16	0.4	0.388 ± 0.005	0.103	0.096	0.102	0.389 ± 0.004	0.101	0.093	0.100	0.392 ± 0.005	0.101	0.103	0.101
0.1	0.36	0.6	0.594 ± 0.005	0.101	0.092	0.101	0.591 ± 0.005	0.105	0.084	0.104	0.601 ± 0.004	0.100	0.101	0.100
0.1	0.64	0.8	0.767 ± 0.005	0.112	0.079	0.107	0.766 ± 0.005	0.116	0.068	0.111	0.795 ± 0.004	0.098	0.099	0.098
0.3	0.04	0.2	0.200 ± 0.003	0.059	0.061	0.059	0.201 ± 0.003	0.059	0.060	0.059	0.200 ± 0.003	0.060	0.061	0.060
0.3	0.16	0.4	0.399 ± 0.003	0.060	0.060	0.060	0.403 ± 0.003	0.060	0.059	0.060	0.402 ± 0.003	0.060	0.059	0.060
0.3	0.36	0.6	0.597 ± 0.003	0.060	0.057	0.059	0.602 ± 0.003	0.059	0.057	0.059	0.599 ± 0.003	0.059	0.057	0.059
0.3	0.64	0.8	0.796 ± 0.003	0.057	0.052	0.057	0.800 ± 0.003	0.058	0.051	0.058	0.802 ± 0.002	0.055	0.054	0.055
0.5	0.04	0.2	0.195 ± 0.002	0.048	0.046	0.048	0.199 ± 0.002	0.045	0.047	0.045	0.198 ± 0.002	0.045	0.047	0.045
0.5	0.16	0.4	0.393 ± 0.002	0.049	0.045	0.049	0.402 ± 0.002	0.045	0.045	0.045	0.399 ± 0.002	0.045	0.045	0.045
0.5	0.36	0.6	0.596 ± 0.002	0.047	0.043	0.047	0.603 ± 0.002	0.043	0.043	0.043	0.599 ± 0.002	0.042	0.043	0.042
0.5	0.64	0.8	0.797 ± 0.002	0.042	0.039	0.041	0.801 ± 0.002	0.039	0.039	0.039	0.798 ± 0.002	0.039	0.039	0.039
Data Simulated from Additive & Non-Additive Model														
0.1	0.04	0.2	0.198 ± 0.004	0.091	0.089	0.091	0.198 ± 0.004	0.092	0.085	0.092	0.198 ± 0.004	0.092	0.092	0.092
0.1	0.16	0.4	0.401 ± 0.004	0.098	0.087	0.098	0.401 ± 0.004	0.092	0.082	0.092	0.407 ± 0.004	0.093	0.091	0.092
0.1	0.36	0.6	0.597 ± 0.004	0.090	0.085	0.090	0.596 ± 0.004	0.093	0.077	0.093	0.604 ± 0.004	0.089	0.090	0.089
0.1	0.64	0.8	0.776 ± 0.004	0.096	0.079	0.093	0.774 ± 0.004	0.102	0.066	0.099	0.794 ± 0.004	0.090	0.088	0.090
0.3	0.04	0.2	0.200 ± 0.002	0.055	0.054	0.055	0.204 ± 0.002	0.054	0.054	0.053	0.200 ± 0.002	0.053	0.053	0.053
0.3	0.16	0.4	0.401 ± 0.002	0.053	0.053	0.053	0.406 ± 0.002	0.053	0.052	0.053	0.400 ± 0.002	0.053	0.052	0.053
0.3	0.36	0.6	0.598 ± 0.002	0.051	0.051	0.051	0.605 ± 0.002	0.052	0.050	0.052	0.598 ± 0.002	0.051	0.050	0.051
0.3	0.64	0.8	0.801 ± 0.002	0.045	0.046	0.045	0.805 ± 0.002	0.048	0.045	0.048	0.802 ± 0.002	0.045	0.047	0.045
0.5	0.04	0.2	0.199 ± 0.002	0.043	0.044	0.043	0.205 ± 0.002	0.042	0.042	0.042	0.200 ± 0.002	0.041	0.041	0.041
0.5	0.16	0.4	0.398 ± 0.002	0.042	0.042	0.042	0.407 ± 0.002	0.041	0.040	0.041	0.399 ± 0.002	0.040	0.039	0.040
0.5	0.36	0.6	0.599 ± 0.002	0.036	0.038	0.036	0.608 ± 0.002	0.036	0.037	0.035	0.598 ± 0.002	0.035	0.037	0.035
0.5	0.64	0.8	0.800 ± 0.001	0.032	0.033	0.032	0.808 ± 0.001	0.033	0.033	0.032	0.801 ± 0.001	0.032	0.032	0.032