ACCOUNTING FOR BREEDING VALUES, HETEROGENEOUS VARIANCES AND MATERNAL EFFECTS IN ESTIMATING SELFING DEPRESSION FOR INDIVIDUAL PEDIGREES

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ABSTRACT

Inbreeding depression (ID) has been estimated for populations and individual pedigrees in many tree species by comparing the mean performance of selfed progeny with related outcrossed progeny. The traditional use of outcrossed progeny as the reference population ($F = 0$) can introduce bias into the estimation of ID for individual pedigrees as well as for populations due to unequal contributions of parental breeding values to selfed and outcrossed progeny. In addition, maternal effects and heterogeneous variances among selfed and outcrossed families may further distort estimates of population and individual ID.

A general linear genetic model for estimating ID in selfing experiments is proposed that adjusts for unequal contributions of breeding values, data imbalance, maternal effects and heterogeneous variances. Under the linear genetic model, four methods to estimate ID for individual parents are proposed that estimate genotypic values of the parents in four different ways. These methods are applied to a numerical data set to illustrate four progressive adjustments in estimating ID for individual parents: (1) adjusting for breeding values only, (2) for both breeding values and heterogenous variances between mating types, (3) for both breeding values and heterogeneous variances among families and (4) for breeding values, heterogeneous variances among families and maternal effects simultaneously.

Keywords: Inbreeding depression, statistical analysis, maternal effect, heterogeneous variance, breeding value

INTRODUCTION

The most striking observed consequence of inbreeding in forest trees is the typical reduction of the mean phenotypic value of quantitative traits. In tree improvement programs, inbreeding may arise when selfing or mating among relatives occurs in open-pollinated families collected from the wild (SHAW & ALLARD 1982) or in seed orchards (MORAN et al. 1980, ADAMS & BIRKES 1989), or when the size of the breeding population is small either initially or due to advancement of the breeding population. Deliberate inbreeding has also been suggested in tree species to purge deleterious alleles, to increase frequency of favourable alleles, and to enhance selection efficiency (BARKER & LIBBY 1974, LINDGREN 1975, WU et al. 1998a). Information on inbreeding depression (ID) could be used to optimize management of advanced breeding populations. ID has been estimated in many important conifer species for many traits, from seed production (FRANKLIN 1970, KOSKI 1971, BRAMLETT & POPHAM 1971,

BRAMLETT & PEPPER 1974, GRIFFIN & LINDGREN 1985), growth traits (ERIKSSON et al. 1973, SORENSEN & MILES 1982, MATHESON et al. 1995), growth curves (WU et al. 1998b) to fecundity (PAWSEY 1964, RUDOLPH 1981 and DUREL et al. 1996). Almost all inbreeding experiments in conifers resulted in ID, and the ID was very severe in many cases (WILLIAMS & SAVOLAINEN 1996).

ID is usually treated as a population concept: a population with an inbreeding coefficient F has a mean genotypic value M_F expressed as

$$M_F = M_0 - 2F \sum_{i=1}^{n} d_i\, p_i\, q_i \qquad [1]$$

where p_i is the frequency of one of two alternate alleles in the population at the ith gene ($q_i = 1 - p_i$), d_i represents the dominance level of the ith gene, M_0 is the population mean before inbreeding, under assumptions that the trait controlled by n loci and there is no epistasis (FALCONER & MACKAY 1996). By this formulation, inbreeding depression is quantified by $M_0 - M_F =$

$2F\sum d_i\,p_i\,q_i$. An individual can be regarded as a special case of the population with $p_i = q_i = 0.5$ for a heterozygous locus.

In practical inbreeding experiments, ID is usually estimated by a simple measure expressed as:

$$ID = 1 - S/O \qquad [2]$$

where S represents the performance of selfs and O is the performance of outbreds, or by regression of trait performance on the level of inbreeding:

$$Y = \alpha + \beta\,(F) + \varepsilon \qquad [3]$$

where Y represents the phenotypic value, α the intercept (estimated population mean when $F = 0$), β the regression line or slope, F the inbreeding coefficient, and ε the residual error term. For selfing experiments, equation (3) produces ID = $-0.5\ \beta/\alpha$, which is equivalent to equation 2.

By its definition in equation 1, ID of an individual tree in a selfing experiment is ideally estimated by comparing the performances of the parent and its selfed offspring. However, in tree species, ID is usually estimated by comparing the performances of selfed or other mildly inbred progeny (such as progeny from matings among half-sibs or full-sibs) with related outcrossed progeny using equation 2 (SNYDER 1972, ERIKSSON et al. 1973, WILCOX 1983, SORENSON 1997), or using equation 3 (ANDERSSON et al. 1974, SNIEZKO & ZOBEL 1988, MATHESON et al. 1995, DUREL et al. 1996).

The direct use of outcrossed progeny as the reference (base) population (with inbreeding coefficient $F = 0$) can introduce biases into the estimation of ID for individual pedigrees and populations. For example, in selfing experiments, the use of outcrossed offspring could introduce bias owing to differential contributions of parental breeding values to the selfed and outcrossed progeny means (BURDON & RUSSELL 1998). In selfing experiments with tree species, half-sib and full-sib crosses are usually used as outcrossed reference populations (SNYDER 1972, ERIKSSON et al. 1973, RUDOLPH 1981, MATHESON et al. 1995, DUREL et al. 1996, WU et al. 1998a). If half-sibs are used as outcrossed progenies, then only half the additive breeding value of the common parent contributes to the mean of outcrossed progeny compared with a full contribution of parental breeding value to the mean of selfed progeny. If full-sibs are used as the outcrossed progeny, again only half the parental breeding value contributes to the mean of the outcrossed progeny in contrast with full contribution to the mean of the selfed progeny. However, in the latter case, additional bias can be introduced if the

breeding value of the mating partner contrasts sharply with that of the selfed parent. The use of mildly inbred progeny (inbreeding level lower than selfing, e.g. matings among half-sibs and full-sibs) as inbreds can also introduce extra biases in estimating ID. Here, the bias may be introduced if the mating partners for the outcrosses and the inbred crosses are different or if the contribution of the mating partners to the outcrosses and inbreds are unequal.

Bias in the estimation of ID for individual parents may also carry into the estimation of ID for the whole population if the unequal contribution of breeding values does not cancel out at the population level. This has prompted BURROWS and ASKEW (1982) to introduce a balanced design for estimating ID at the population level.

It is meaningful to estimate ID for individual pedigrees only if there is significant interaction between pedigree and inbreeding level (i.e. if some parants display more ID than others). This pedigree by inbreeding level interaction was traditionally addressed through the linear model $Y_{ijk} = \mu + f_i + m_j + fm_{ij} + w_{ijk}$ and by testing the significance of fm_{ij} term, where f_i is the effect of pedigree (seed parent), m_j is that of mating types (inbred and non-inbred), fm_{ij} is that of the interaction between seed parent and mating type (inbreeding level), and w_{ijk} is the residual effect (SORENSEN & MILES 1982, LUNDKVIST et al. 1987, GRIFFIN & COTTERILL 1988, MATHESON et al. 1995, Shortt et al. 1996, DUREL et al. 1996). However, such testing for fm_{ij} in the traditional linear model will introduce a spurious interaction owing to the differential contributions of breeding values to f_i (BURDON & RUSSELL 1998). Thus, there is a need to develop a proper test for significant interaction between pedigree and mating type before estimating ID for individual pedigrees.

There are other possible biases in estimating ID (WILLIAMS & SAVOLAINON 1996, BURDON & RUSSELL 1998). Maternal effects and heterogeneous variances among mating types or families are two of the more obvious complications in estimating population and individual ID.

To overcome differential contributions of breeding values in estimating ID at the population level, BURROWS and ASKEW (1982) introduced a linear model to account for the imbalance of family additive coefficients of relationship. But the interaction between pedigree and inbreeding level was not tested. BURDON and RUSSELL (1998) proposed an alternative model and procedure for selfing experiments. In their model, a large panmictic population was regarded as a normal baseline population from which baseline parameters are estimated. Selfed families were regarded as deviants from the norm of the outcrossed families, thus ID for

individual pedigrees was estimated from the difference in the parameters, which were estimated from two separate models (outcross and self). However, there are some statistical disadvantages to addressing outcrossing as a typical norm. Since two models are separately used in estimating the effect of inbreeding depression, the estimates are not least-squares estimates when the data are unbalanced (e.g. unbiased minimum variance estimates, BLUE). The recommended procedure also requires several independent steps: using different models for self, outcrossed and combined family, and using ANCOVA (analysis of covariance) for adjusting for maternal effect.

In this paper, a more general linear genetic model is proposed that adjusts for unequal contributions of breeding values when estimating ID from selfing experiments with half-sibs used as the outcrossed reference population ($F = 0$). The model can also account for data imbalance, maternal effect and heterogeneous variance simultaneously. A more generalised case to deal with pair-crosses among unrelated parents as the reference population ($F = 0$) and use of mildly inbred material (e.g. mating among half-sibs, full-sibs) as inbreds will be dealt with in a later paper.

MODEL AND THEORY

When half-sib families are used as an outcrossed reference population and selfed progeny as inbred material, a linear model to estimate ID while accounting for unequal GCA effects could be constructed as

$$Y_{ijk} = \mu + r_{ij}\, g_i + m_j + I_{ij} + w_{ijk} \qquad [4]$$

where μ is the grand mean, g_i is the general combining ability (GCA) for pedigree (seed parent) i, m_j is the effect of mating type (j = o for half-sib outcross and j = s for self), I_{ij} is the interaction between seed parent (represented by g_i) and inbreeding level (mating type m_j), w_{ijk} is the residual effect with variance σ_w^2, and r_{ij} is a coefficient appropriate to the degree of relationship ($r_{ij} = 1$ when j = o and $r_{ij} = 2$ when j = s). With this formulation, both selfing and outcrossing families can be represented with a single model and information from both mating types is used for estimating GCA and inbreeding depression for a population as well as for individual pedigrees. In addition, a proper statistical test for the interaction between seed parent and inbreeding level can be constructed. The interaction (I_{ij}) in this model represents a function of the interaction between the GCA of the seed parent and the mating type, in contrast with the simplistic interaction of seed parent and mating type.

Assuming balanced data, the exact sum of squares

for testing the interaction of the GCA and mating types in the above linear model 4 for a parents and n progenies of each family could be derived from an ordinary least-squares analysis as

$$\frac{4n}{5}\sum_{i=1}^{a}(\bar{y}_{io\cdot} - \bar{y}_{\cdot o\cdot})^2 + \frac{n}{5}\sum_{i=1}^{a}(\bar{y}_{is\cdot} - \bar{y}_{\cdot s\cdot})^2 - \frac{4n}{5}\sum_{i=1}^{a}(\bar{y}_{io\cdot} - \bar{y}_{\cdot o\cdot})([5]$$

where,

$$\bar{y}_{io\cdot} = \frac{\sum_{k=1}^{n} y_{iok}}{n}, \quad \bar{y}_{is\cdot} = \frac{\sum_{k=1}^{n} y_{isk}}{n}, \quad \bar{y}_{\cdot o\cdot} = \frac{\sum_{i=1}^{a} \bar{y}_{io\cdot}}{a}, \quad \bar{y}_{\cdot s\cdot} = \frac{\sum_{i=1}^{a} \bar{y}_{is\cdot}}{a}$$

with a dot representing summation over the subscript and a bar representing the mean over the summation. With the usual parameter constraints of linear model

$$\sum_{i=1}^{a} g_i = 0, \quad \sum_{j=o}^{s} m_j = 0, \quad and \quad \sum_{i=1}^{a} I_{ij} = 0,$$

for each j and $\sum_{j=o}^{s} I_{ij} = 0$, for each i,

the best linear unbiased estimate (BLUE) for GCA effects (g_i), mating type effects (m_o and m_s) and the interaction effect I_{ij} can be estimated as follows:

$$\hat{g}_i = \frac{1}{9}(2\,\bar{y}_{i\cdot}'' - 2\,\bar{y}_{\cdots}'' + \bar{y}_{i\cdot}' - \bar{y}_{\cdots}') \qquad [6]$$

where

$$\bar{y}_{i\cdot}' = \bar{y}_{io\cdot} - \bar{y}_{is\cdot}, \quad \bar{y}_{i\cdot}'' = \bar{y}_{io\cdot} + 2\bar{y}_{is\cdot}, \quad \bar{y}_{\cdots}' = \frac{\sum_{i=1}^{a}\bar{y}_{i\cdot}'}{a} \quad \bar{y}_{\cdots}'' = \frac{\sum_{i=1}^{a}\bar{y}_{i\cdot}''}{a}$$

Upon simplification, equation 6 becomes

$$\hat{g}_i = \frac{1}{3}(\bar{y}_{io\cdot} - \bar{y}_{\cdot o\cdot} + \bar{y}_{is\cdot} - \bar{y}_{\cdot s\cdot}) \qquad [7]$$

for g_1 to g_a, and

$$\hat{I}_{ij} = \frac{1}{9}(5\,\bar{y}_{i\cdot}' - 5\,\bar{y}_{\cdots}' + \bar{y}_{i\cdot}'' - \bar{y}_{\cdots}'') \qquad [8]$$

or alternatively, interaction effects can be estimated as

$$\hat{I}_{ij} = \frac{1}{3}[2(\bar{y}_{io\cdot} - \bar{y}_{\cdot o\cdot}) - (\bar{y}_{is\cdot} - \bar{y}_{\cdot s\cdot})] \qquad [9]$$

for I_{lo} to I_{ao}, and the mating effect for half-sibs is estimated as

$$\hat{m}_o = \frac{1}{(2*a)}\sum_{i=1}^{a}(\bar{y}_{io\cdot} - \bar{y}_{is\cdot}) = \frac{1}{2}\bar{y}_{\cdots}' \qquad . \qquad [10]$$

From these derivations, ID for the whole population can be estimated as

$$\hat{m}_o - \hat{m}_s = \bar{y}_{.o.} - \bar{y}_{.s.} \qquad [11]$$

and correspondingly, for the ith individual seed parent, ID can be estimated as

$$\hat{m}_o - \hat{m}_s + \hat{I}_{io} - \hat{I}_{is} \qquad [12]$$

where $I_{io}-I_{is}$ represents the individual contribution to ID after accounting for the population contribution. Alternatively, ID for individual seed parent can be estimated from raw data as

$$(\bar{y}_{.o.} - \bar{y}_{.s.}) + \frac{2}{3}[2(\bar{y}_{io.} - \bar{y}_{.o.}) - (\bar{y}_{is.} - \bar{y}_{.s.})]. \qquad [13]$$

In most publications, the ID was more conveniently expressed as a proportion by the equation 2. According to our derivations of various components, the ID of an individual seed parent can be estimated in several ways according to choice of baseline:

1. Based on the additive genetic value of the parent tree only (method 1)

$$1 - \frac{\mu + 2g_i - (m_o - m_s) - (I_{io} - I_{is})}{\mu + 2g_i}. \qquad [14]$$

2. Based on half the additive genetic value of the parent tree only (method 2)

$$1 - \frac{\mu + g_i - (m_o - m_s) - (I_{io} - I_{is})}{\mu + g_i}. \qquad [15]$$

Method 2 is the closest to the traditional calculation method: e.g. using open-pollinated family mean as the outbred reference ($F = 0$).

3. Based on the additive genetic value and the non-additive genetic effect (m_o and I_{io}) of the parent tree (method 3)

$$1 - \frac{\mu + 2g_i + m_s + I_{is}}{\mu + 2g_i + m_o + I_{io}}. \qquad [16]$$

4. Based on half the additive genetic value of the parent tree and the non-additive genetic effect (method 4)

$$1 - \frac{\mu + g_i + m_s + I_{is}}{\mu + g_i + m_o + I_{io}}. \qquad [17]$$

Method 3 and 4 are better representations of true inbreeding depression for parent trees because they include both additive and non-additive effects when estimating the genotypic value of the parent tree. In particular, method 3 is likely the closest to the true definition of inbreeding depression for an individual seed parent, since the value of the denominator is the closest to the true genotypic value of the seed parent. This is because $2g_i$ accounts for the additive genetic value of the parent tree and $m_o + I_{io}$ accounts for non-additive genetic effects of the seed parent (dominance and/or epistasis).

Model 4 can also be extended to accommodate unbalanced data (e.g. $n_{io} \neq n_{is}$), heterogeneous residual variances among half-sib and self material, and maternal effects such as seed weight and/or germination rate (assuming seed weight and germination rate are purely determined by maternal effects).

To incorporate data imbalance, the means used for estimating SSQ_I and other parameters are replaced by

$$\bar{y}_{io.} = \frac{\sum_{k=1}^{n_{io}} y_{iok}}{n_{io}}, \quad \bar{y}_{is.} = \frac{\sum_{k=1}^{n_{is}} y_{isk}}{n_{is}}, \quad \bar{y}_{.o.} = \frac{\sum_{i=1}^{a} \bar{y}_{io.}}{a}, \quad \bar{y}_{.s.} = \frac{\sum_{i=1}^{a} \bar{y}_{is.}}{a}$$

and SSQ_I is then estimated by

$$SSQ_I = \frac{4}{5}(\sum_{i=1}^{a} n_{io} \bar{y}_{io.}^2 - n_{.o} \bar{y}_{.o.}^2) + \frac{1}{5}(\sum_{i=1}^{a} n_{is} \bar{y}_{is.}^2 - n_{.s} \bar{y}_{.s.}^2)$$
$$\cdot [\frac{2}{5}\sum_{i=1}^{a} n_{is} \bar{y}_{is.} \bar{y}_{io.} + \frac{2}{5}\sum_{i=1}^{a} n_{io} \bar{y}_{io.} \bar{y}_{is.} - \frac{2}{5}\sum_{i=1}^{a}(n_{.o} + n_{.s}) \bar{y}_{.o.} \bar{y}. \qquad [18]$$

where n_{io} and n_{is} are the numbers of outcrossed and selfed progeny observed from the ith parent and

$$n_{.o} = \sum_{i=1}^{a} n_{io}, \quad n_{.s} = \sum_{i=1}^{a} n_{is}.$$

To incorporate heterogeneous variances between the residuals of half-sib (σ_{wo}^2) versus selfs (σ_{ws}^2) in the linear model 4, a weighted least squares analysis of model 4 with a weighting matrix V is used. The residual variances can be expressed as Var $(e) = IV$,

$$V = \begin{bmatrix} \dfrac{1}{\sigma_{wo}^2} & 0 \\ 0 & \dfrac{1}{\sigma_{ws}^2} \end{bmatrix}$$

where and I is an identity matrix with dimension of a*n; or alternatively we could use the following 2a × 2a matrix of V with the dimension of I equal to n

$$V = \begin{bmatrix} \dfrac{1}{\sigma_{wlo}^2} & 0 & 0 & \ldots & 0 & 0 \\ 0 & \dfrac{1}{\sigma_{wls}^2} & 0 & \ldots & 0 & 0 \\ & & \ldots\ldots\ldots\ldots & & \\ 0 & 0 & 0 & \ldots & \dfrac{1}{\sigma_{wao}^2} & 0 \\ 0 & 0 & 0 & \ldots & 0 & \dfrac{1}{\sigma_{was}^2} \end{bmatrix}$$

where σ_{wio}^2 and σ_{wis}^2 represent the residual variances of the outcrossed and selfed families for the ith parent. The former assumes the same residual variance for the same mating type while the latter adjusts the residual variance for both mating type and individual pedigree. Adjusting for both mating type and individual pedigree is more desirable because the residual variance may be different not only between mating types but also among pedigrees. In practice, estimated residual variances for σ_{wo}^2, σ_{ws}^2, σ_{wio}^2 and σ_{wis}^2 are usually used for the V matrix, the use of estimated residual variances will introduce some bias if family size is small. Since V is a non-singular matrix, $b' = [\mu, g_1, g_2, \ldots g_{(a-1)}, m_o, I_{1o}, I_{2o}, \ldots I_{(a-1)o}]$ can be estimated by solving the normal equations (SEARLE 1971)

$$\hat{b} = (X'(IV)^{-1}X)^{-1}X'(IV)^{-1}Y \qquad [19]$$

where Y is the vector for individual observations and the incidence matrix X (with number of rows equal to number of observations and number of columns equal to 2a) is as shown below

$$b' = [\mu \quad g_1 \quad g_2 \quad \ldots \quad g_{(a-1)} \quad m_o \quad I_{1o} \quad I_{2o} \ldots \quad I_{(a-1)o}]$$

$$X = \begin{bmatrix} 1 & 1 & 0 & \ldots & 0 & 1 & 1 & 0 & \ldots & 0 \\ 1 & 2 & 0 & \ldots & 0 & -1 & -1 & 0 & \ldots & 0 \\ 1 & 0 & 1 & \ldots & 0 & 1 & 0 & 1 & \ldots & 0 \\ 1 & 0 & 2 & \ldots & 0 & -1 & 0 & -1 & \ldots & 0 \\ & & & \ldots\ldots\ldots\ldots\ldots & & & \\ 1 & -1 & -1 & \ldots & -1 & 1 & -1 & -1 & \ldots & -1 \\ 1 & -2 & -2 & \ldots & -2 & -1 & 1 & 1 & \ldots & 1 \\ & & & \ldots\ldots\ldots\ldots\ldots & & & \end{bmatrix}$$

Similarly, the sum of squares for interaction SSQ_I under heterogeneous variances can be derived by fitting the full model 4 and the following sub-model 20 according

to the usual least-squares method for unbalanced data:

$$Y_{ijk} = \mu + r_{ij}\, g_i + m_j + w_{ijk} \qquad [20]$$

Thus, if the reduction of the sum of squares for fitting model 4 is written as

$$R(\mu, g, m, I) = b'X'(IV)^{-1}Y, \qquad [21]$$

then the reduction of the sum of squares for fitting submodel 20 is

$$R(\mu, g, m) = b_{sub}' \, X_{sub}'(IV)^{-1}Y, \qquad [22]$$

where X_{sub} is similar to X except that all interaction columns were deleted. The sum of squares for the interaction I_{ij} (SSQ_I) is the difference between the two reductions

$$R(I|\mu, g, m) = R(\mu, g, m, I) - R(\mu, g, m). \qquad [23]$$

One simple way to adjust for maternal effects is to set maternal effect as a covariate in the linear model 4. For example, if seed weight is purely determined by maternal genotypes, the seed weight x_{ijk} for the kth seed of the jth mating type from the ith parent can be incorporate into the linear model as

$$Y_{ijk} = \mu + x_{ijk} + r_{ij}\, g_i + m_j + I_{ij} + w_{ijk}. \qquad [24]$$

Similarly, model 4, 20 and 24 can be combined to account for unequal contribution of breeding values, heterogeneous variances, and maternal effects simultaneously if the error matrix $Var(e) = IV$ is defined accordingly.

EXAMPLE AND DISCUSSION

A simulated numerical example of three seed parents (a = 3) with half-sib matings as an outcrossed reference population (designated as O), selfed progeny as inbreds (designated as S) and two to four trees (n_{io} or n_{is} = 2, 3 or 4) for each family is generated to demonstrate the use of the model 4, 20, and 24 to test the seed parent by mating type interaction and to estimate ID for individual seed parents. The raw data for computation are presented in Appendix 1.

Calculations of five cases representing unadjusted (Case 1) and four progressive adjustments: adjusting for breeding values only (Case 2), adjusting for breeding values and heterogeneous variances between mating types (Case 3), adjusting for breeding values and heterogeneous variances among families (Case 4), and adjusting for breeding values, heterogeneous variances

among families, and maternal effects simultaneously (Case 5) are presented along with the unadjusted model. In this example, seed weight effect was regarded as pure maternal effect and used as a covariate in the model, however, cautious should be exercised since seed weight may also be affected by self depression. SAS IML and GLM Procedures were used for the computation (SAS Institute Inc. 1989).

Results from ANOVA (hypothesis tests of interactions and pair-wise contrasts for testing the interaction of any two parents with mating type) for the four adjusted cases and the unadjusted case are listed in Table 1 and the corresponding parameter estimates are listed in Table 2.

The estimates of ID for population were the same (1– (12.45–2.01)/(12.45+2.01) = 0.278 = 27.8%) for unadjusted case (Case 1), Case 2, Case 3 and Case 4. Adjusting for breeding values, heterogeneous variances, and maternal effects simultaneously (Case 5) produced a slightly higher ID for the population (ID = 28.28%).

Results from the unadjusted case indicated that there were significant differences ($P < 0.01$) among parents and mating types (half-sib and self) as well as interactions between parent and mating type (Table 1). Among three independent contrasts of pair-wise parental combinations, interactions between parents 1 & 2 and mating type, and between parents 2 & 3 and mating type were highly significant ($P < 0.01$), and interaction between parent 1 & 3 and mating type was also significant ($P < 0.05$). After adjusting for GCA only, the mean squares for GCA*mating type interaction was reduced, although it was still significant. However, the interaction between parents 2 & 3 and mating type

became non-significant ($P > 0.05$) after adjusting for GCA effect. After adjusting for GCA and heterogeneous variances simultaneously, the mean squares for GCA, and mating type were both increased. The interaction between parents 2 & 3 and mating type was also non-significant ($P > 0.05$) as in the Case 2 model. Further adjustment for seed weight significantly reduced the mean squares for GCA effect, mating type, and their interaction (Table 1).

In comparison with the unadjusted model (see note for Table 1), adjustment for differential contribution of breeding values in half-sibs and selfed progeny reduced the range of parental component estimates from 7.25 (–4.351 to 2.903) to 4.836 (–2.901 to 1.935) (GCA component in the adjusted model) and the adjustment also had a significant effect on the estimate of the interaction component (Table 2). However, adjusting for GCA did not have any impact on the estimate of the mating-type effect (m_o, m_s) or the population ID, as expected. Further adjustments for heterogeneous variances (among mating types or families) had no impact at all on all estimated effects versus adjustment for GCA only (Case 2) although in both cases (Case 3 and Case 4) the mean squares for GCA and mating type were increased (Table 1). This is because the same weight was used for individuals within the same mating type (Case 3) or the same family (Case 4). However, adjusting for heterogeneous variances between mating types or among families did affect standard errors of the estimates of GCA, mating-type and interaction components (Table 2). Further adjustment for maternal effects, in addition to GCA and heterogeneous variances not only altered the estimates of the GCA and

Table 1. ANOVA for five models for the analysis of inbreeding depression: unadjusted (Case 1), adjusted for breeding value only (Case 2), for breeding value and heterogenous variances between mating type (Case 3), for breeding value and heterogenous variances among families (Case 4) and for breeding value, heterogenous variance among families and maternal effect (Case 5).

Sources of variation	DF	Case 1		Case 2		Case 3		Case 4		Case 5	
		MS[a]	P>F	MS	P>F	MS	P>F	MS	P>F	MS	P>F
GCA	2	89.96	0.001	89.96	0.001	139.57	0.001	134.54	0.001	59.53	0.001
Mating-type (M)	1	69.60	0.001	69.60	0.001	108.31	0.001	91.43	0.001	60.97	0.001
GCA*M	2	37.40	0.001	16.76	0.001	24.39	0.001	15.66	0.001	13.42	0.001
Contrast											
P1&P2*M	1	34.16	0.001	21.55	0.001	31.23	0.001	28.25	0.001	25.39	0.001
P1&P3*M	1	4.61	0.036	27.19	0.001	39.53	0.001	25.70	0.001	20.39	0.001
P2&P3*M	1	15.98	0.001	0.003	0.949	0.005	0.952	0.007	0.931	0.010	0.923
Residual	12	0.83		0.83		1.33		1.00		1.03	(D.F.=11)

[a] For the unadjusted model ($y_{ijk} = \mu + p_i + m_j + l_{ij} + w_{ijk}$), the mean squares (MS) for GCA represents the mean squares due to parent effect and the MS for GCA*M represents the mean squares for Parent*M interaction

Table 2. Estimated parameters (EST) and standard error (SE) from the five models: unadjusted (Case 1), adjusted for breeding value only (Case 2), for breeding value and heterogenous variances between mating types (Case 3), for breeding value and heterogenous variances among families (Case 4) and for breeding value, heterogenous variance among families and maternal effect (Case 5).

Parameter	Case 1		Case 2		Case 3		Case 4		Case 5	
	EST[a]	SE	EST	SE	EST	SE	EST	SE	EST	SE
μ	12.451	0.219	12.451	0.218	12.451	0.223	12.451	0.210	11.579	1.074
g_1	1.449	0.307	0.966	0.204	0.966	0.207	0.966	0.209	0.785	0.302
g_2	2.903	0.325	1.935	0.216	1.935	0.221	1.935	0.208	1.916	0.212
g_3	–4.351	0.297	–2.901	0.198	–2.901	0.202	–2.901	0.178	–2.701	0.301
m_o	2.007	0.219	2.007	0.219	2.007	0.222	2.007	0.210	1.907	0.244
m_s	–2.007	0.219	–2.007	0.219	–2.007	0.222	–2.007	0.210	–1.907	0.244
l_{11}	1.593	0.307	2.076	0.329	2.076	0.346	2.076	0.372	1.994	0.390
l_{12}	–1.593	0.307	–2.076	0.329	–2.076	0.346	–2.076	0.372	–1.994	0.390
l_{21}	–1.986	0.325	–1.019	0.359	–1.019	0.378	–1.019	0.277	–1.020	0.281
l_{22}	1.986	0.325	1.019	0.359	1.019	0.378	1.019	0.277	1.020	0.281
l_{31}	0.393	0.297	–1.057	0.325	–1.057	0.343	–1.057	0.302	–0.974	0.322
l_{32}	–0.393	0.297	1.057	0.325	1.057	0.343	1.057	0.302	0.974	0.322
$\mu + m_o$	14.458		14.458		14.458		14.458		13.486	
Population	4.014		4.014		4.014		4.014		3.814	
%	27.76		27.76		27.76		27.76		28.28	

[a] For the unadjusted model, the parameters of g_1, g_2 and g_3 represent the parental effect $p_1, p_2,$ and p_3: i.e. effect of the parent rather than the GCA effect.

Table 3. Estimated ID(%) for individual parents based on five models: unadjusted (Case 1), adjusted for breeding value only (Case 2), for breeding value and heterogenous variances between mating types (Case 3), for breeding value and heterogenous variances among families (Case 4) and for breeding value, heterogenous variances among families and maternal effect (Case 5) according to four calculation methods[a]

Method	Parent	Unadjusted	Case 2, Case 3, Case 4	Case 5
Method 1	P_1	51.80	56.54	59.34
	P_2	0.27	12.11	11.51
	P_3	59.26	28.58	30.21
Method 2	P_1	Same as method 1	60.86	63.31
	P_2		13.74	13.15
	P_3		19.90	21.02
Method 3	P_1	41.14	44.08	45.76
	P_2	26.11	11.42	10.88
	P_3	38.23	25.00	26.24
Mehod 4	P_1	Same as method 3	46.66	47.97
	P_2		12.85	12.33
	P_3		18.10	19.02

[a] See text for computational formula of the four methods.

interaction effects, but also the estimates of the mating type effects (m_o, m_s) and the overall mean (μ = 13.86) in comparison with the previous four situations (unad-justed, adjusted for breeding values only, and adjusted for breeding values and heterogeneous variances among mating types or families). The adjustment for maternal

effect increased the population ID from 27.8% to 28.3% (Table 2).

The choice of methods for calculation and adjustment for breeding value, heterogeneous variances and maternal effects had significant impacts on estimates of individual ID (Table 3). For the unadjusted model, Methods 1 and 2 (regarded as group 1 here) produced the same estimates and Methods 3 and 4 (regarded as group 2) did as well. But ID estimates were different between the two groups of methods, the former group had higher estimates of ID for parent 1 and 3 and lower estimate of ID for parent 2. This is because the former group (group 1) only used the additive genetic effect of a parent as the baseline while the latter group used the additive and non-additive genetic effects as the baseline for inbreeding depression. For example, in Case 2, ID estimates from Methods 1 and 2 were higher for parent 1 than from Methods 3 and 4, but ID estimates were similar for parent 2 among the four methods.

The effects of different adjustments on individual ID can be illustrated using Method 1. Without adjustment, ID for parent 2 was close to 0 (0.3 %), and adjustments for breeding values, or for GCA, heterogeneous variances and maternal effects simultaneously had ID estimates of 12.1%, and 11.5%, respectively. Progressive adjustments increased the estimated ID for parent 1 from 51.8 % to 56.5 %, and 59.3 %, respectively, while progressive adjustment for parent 3 reduced the estimated ID from 59.3 % to 28.6 %, and 30.2 %, respectively (Table 3).

Estimation of inbreeding depression for a population is not biased if relative balance of individual ancestral contributions at different inbreeding levels is achieved as in this example. BURROWS and ASKEW (1982) presented an orthogonal mating design for balancing the contribution of breeding value at all inbreeding levels. But the design requires a minimum of 12 progeny families derived from 8 grandparents, and most existing inbreeding trials in forest trees do not conform to such a design structure. Small deviation from orthogonality may not have a huge effect on estimated population ID as much of the parental breeding values may cancel out at the population level if a good balance of pedigree contributions is achieved. However, estimating ID for individual pedigrees is more complex, even with the orthogonal mating design proposed by BURROWS and ASKEW (1982), because unequal contribution of breeding values from mating partners at different inbreeding levels may not cancel out as we demonstrated in this example. In principle, comparing a parent with its selfed offspring is the most precise way to study selfing effects. In doing so, however, rejuvenation of parental material and vegetative propagation of the parent are necessary, among other things.

But this vegetative propagation may introduce some other biases associated with the age and position of the propagule. In addition, it can be difficult to vegetatively propagate mature material in many species.

The advantage of the model proposed in this paper is that it can account for breeding value, heterogeneous variances, and maternal effect simultaneously without the need to invoke the full complexity of, in addition to additivity and dominance, other more complex gene actions and variances associated with inbreeding data. The best linear unbiased estimates can be derived for model parameters and ID can be estimated for the population as well as for individual pedigrees. The model can also be expanded into more complex situations such as where progeny from full-sib matings are used as the outbred reference population, where there are more than two levels of inbreeding and where mildly inbred populations are used.

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Appendix 1. Simulated numerical data for inbreeding (selfing) and outcrossing (half-sib matings) for three seed parents (mating type: S stands for selfing, O stands for half-sib).

No.	Parent	Mating type	Tree no.	Height	Seed weight
1	1	O	1	19.00	6.5
2	1	O	2	16.50	5.8
3	1	O	3	17.00	6.0
4	2	O	1	15.75	4.5
5	2	O	2	15.00	5.3
6	3	O	1	11.50	3.5
7	3	O	2	10.00	3.3
8	3	O	3	10.00	3.4
9	1	S	1	10.40	5.6
10	1	S	2	10.00	5.3
11	1	S	3	10.50	4.7
12	2	S	1	16.75	3.9
13	2	S	2	15.25	4.4
14	2	S	3	14.00	3.7
15	3	S	1	6.00	2.0
16	3	S	2	5.50	2.3
17	3	S	3	5.00	2.1
18	3	S	4	6.30	2.6