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ORIGINAL PAPER

# Genomewide predictions from maize single-cross data

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**Abstract** Maize (*Zea mays* L.) breeders evaluate many single-cross hybrids each year in multiple environments. Our objective was to determine the usefulness of genomewide predictions, based on marker effects from maize single-cross data, for identifying the best untested single crosses and the best inbreds within a biparental cross. We considered 479 experimental maize single crosses between 59 Iowa Stiff Stalk Synthetic (BSSS) inbreds and 44 non-BSSS inbreds. The single crosses were evaluated in multilocation experiments from 2001 to 2009 and the BSSS and non-BSSS inbreds had genotypic data for 669 single nucleotide polymorphism (SNP) markers. Single-cross performance was predicted by a previous best linear unbiased prediction (BLUP) approach that utilized marker-based relatedness and information on relatives, and from genomewide marker effects calculated by ridge-regression BLUP (RR-BLUP). With BLUP, the mean prediction accuracy ($r_{MG}$) of single-cross performance was 0.87 for grain yield, 0.90 for grain moisture, 0.69 for stalk lodging, and 0.84 for root lodging. The BLUP and RR-BLUP models did not lead to $r_{MG}$ values that differed significantly. We then used the RR-BLUP model, developed from single-cross data, to predict the performance of testcrosses within 14 biparental populations. The $r_{MG}$ values within each testcross population were generally low and were often negative. These results were obtained despite the above-average level of linkage disequilibrium, i.e., $r^2$ between adjacent markers of 0.35 in the BSSS inbreds and 0.26 in the non-BSSS inbreds. Overall, our results suggested that genomewide marker effects estimated from maize single crosses are not advantageous (compared with BLUP) for predicting single-cross performance and have erratic usefulness for predicting testcross performance within a biparental cross.

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## Introduction

Genomewide selection (or genomic selection) is a method for marker-assisted selection without quantitative trait locus (QTL) mapping. Genomewide selection uses all available markers instead of only those markers linked to QTL to identify the best individuals in a population (Meuwissen et al. 2001). Our previous studies have shown that genomewide selection is effective within a biparental cross, where genomewide prediction equations are constructed from phenotypic and marker data from the progeny of a cross between two inbreds (e.g., A × B) and are applied to an untested subset of individuals of the same A × B cross in one or more cycles of selection (Bernardo and Yu 2007; Lorenzana and Bernardo 2009; Bernardo et al. 2011).

Commercial maize breeding programs generate large numbers of single crosses that are widely evaluated across multiple environments (Hallauer 1990; Bernardo 1996a). Instead of using phenotypic and marker data from the

progeny of an A × B breeding cross, single-cross hybrid data could be used as a training population for genomewide selection of the best inbreds within the A × B cross. In addition, the genomewide marker effects estimated from single-cross hybrid data could be used to identify the best single crosses prior to testing. In this case, genomewide prediction of single-cross performance needs to be compared to previous approaches used to predict single-cross performance. In particular, the best linear unbiased prediction (BLUP) has previously been found useful for predicting the performance of untested single crosses based on the performance of the tested single crosses and the relatedness between the untested and tested single crosses (Bernardo 1994; 1996a, b). While genomewide predictions of single-cross performance would rely on genomewide marker effects, such previous BLUP approach for predicting single cross performance utilizes any available marker data only to estimate relatedness among parental inbreds (Bernardo 1994). Correlations between predicted and observed maize single-cross performance ranged from 0.42 to 0.76 for grain yield, 0.75 to 0.93 for grain moisture, 0.30 to 0.74 for stalk lodging, and 0.16 to 0.53 for root lodging (Bernardo 1996a). The BLUP approach has since been routinely used in maize breeding programs.

Single-cross hybrid data have two key features that make them attractive as training populations in genomewide selection. First, experimental single crosses comprise elite germplasm in a maize breeding program, and the parental inbreds of single-cross hybrids are the ones used most often in creating A × B biparental populations from which new inbreds are developed (Hallauer 1990). Second, the extensive multi-environment testing of single crosses permits a sampling of a wide array of genotype-environment interactions (Smith et al. 1999). Single-cross data have been successfully used to detect QTL within different heterotic groups in maize (Parisseaux and Bernardo 2004; van Eeuwijk et al. 2010).

Published information is unavailable, however, on the usefulness of single-cross hybrid data for genomewide selection. Our objective was to determine the usefulness of genomewide predictions, based on marker effects estimated from maize single-cross data, for identifying the best untested single crosses and the best inbreds within a biparental cross.

## Materials and methods

### Single-cross phenotypic and SNP data

We considered 479 experimental maize single crosses developed and tested by AgReliant Genetics, LLC. The single crosses were made between 59 Iowa Stiff Stalk Synthetic (BSSS) inbreds and 44 non-BSSS inbreds and had relative maturities of 104–115 days. Each BSSS inbred was crossed with 1–24 non-BSSS inbreds (mean of 8.4). Each non-BSSS inbred was crossed with 1–48 BSSS inbreds (mean of 11.4).

The phenotypic data set was taken from 271 experiments conducted by AgReliant Genetics from 2001 to 2009, with one experiment being defined as a set of hybrids evaluated in multiple locations in a given year. Each of the 271 experiments was conducted at 6–66 locations in the US Corn Belt (Supplementary Fig. 1). The data were highly unbalanced across experiments but, aside from occasional missing plots at individual locations, were balanced within each experiment. The single crosses were evaluated for grain yield, grain moisture, stalk lodging, and root lodging. The number of experiments for each single cross had a mean of 4.75 and a median of 2 (Supplementary Fig. 2). The mean number of locations per experiment was 25 for grain yield and grain moisture, and 23 for stalk and root lodging.

The 103 BSSS and non-BSSS inbreds were genotyped with 768 well-distributed SNP markers used routinely by AgReliant Genetics. We retained 669 SNP markers after screening the marker data against excessive heterozygosity and missing genotypes. All markers were mapped to the IBM2 2008 neighbors map, available on MaizeGDB.org, for the intermated B73 × Mo17 population (Lee et al. 2002). To correct for the expansion of the IBM2 map due to several generations of intermating the B73 × Mo17 population, the map distances between adjacent markers were divided by a factor of 3.8. This factor was determined by comparing the map distances of common markers between the IBM2 2008 neighbors map versus the 1997 and 1999 maps (available on MaizeGDB.org) of a non-intermated B73 × Mo17 population. To characterize linkage disequilibrium (LD), $r^2$ values were calculated between all pairs of markers using Haploview v 4.0 (Barrett et al. 2005).

Some inbreds had a transgenic and a non-transgenic version. As shown later, the presence of transgenes was modeled as a fixed effect. A total of 37 different combinations of transgenes were present in the data set.

### Marker-based estimates of coefficient of coancestry

Marker similarity was calculated among all inbreds as the proportion of SNP loci at which two inbreds were homozygous for the same SNP allele. Marker-based estimates of the coefficient of coancestry ($f_{ij}$) were then calculated among the BSSS inbreds and among the non-BSSS inbreds. Within a heterotic group, the probability that unrelated inbreds had alleles that were alike in state was unknown.

Assuming that (1) the probability of alikeness in state between unrelated inbreds was equal between the BSSS and non-BSSS heterotic groups and that (2) the BSSS inbreds were unrelated to the non-BSSS inbreds, the following procedure was used to estimate $f_{ij}$. Suppose $i$ and $j$ were two inbreds in the same heterotic group. The $f_{ij}$ was estimated as $[S_{ij} - 0.5(S_{i-} + S_{j-})]/[1 - 0.5\ (S_{i-} + S_{j-})]$, where $S_{ij}$ was the marker similarity between $i$ and $j$; $S_{i-}$ was the mean marker similarity between $i$ and all inbreds in the opposite heterotic group; and $S_{j-}$ was the mean marker similarity between $j$ and all inbreds in the opposite heterotic group (Bernardo 1993). Only 1.8 % of the $f_{ij}$ estimates were negative and were set equal to zero.

## BLUP model for predicting single-cross performance

The BLUP model was the same as that described by Bernardo (1996a), except that the current analysis included the fixed effects of transgenes and excluded the effects of check hybrids. Assume that $N$ single crosses were made between $n_1$ BSSS inbreds and $n_2$ non-BSSS inbreds. The single crosses, which included $t$ transgene combinations, were evaluated in $c$ experiments resulting in $p$ data points. The linear model for single-cross performance for a given trait was

$$\mathbf{y} = \mathbf{X_0}\boldsymbol{\beta} + \mathbf{X_1}\boldsymbol{\theta} + \mathbf{Z_1}\mathbf{g_1} + \mathbf{Z_2}\mathbf{g_2} + \mathbf{Z_3}\mathbf{s} + \mathbf{e} \quad (1)$$

where $\mathbf{y} = p \times 1$ vector of observed single-cross performance; $\boldsymbol{\beta} = c \times 1$ vector of fixed effects due to experiments; $\boldsymbol{\theta} = t \times 1$ vector of fixed effects due to transgene combinations; $\mathbf{g_1} = n_1 \times 1$ vector of random effects due to general combining ability (GCA) of BSSS inbreds; $\mathbf{g_2} = n_2 \times 1$ vector of random effects due to GCA of non-BSSS inbreds; $\mathbf{s} = N \times 1$ vector of random effects due to specific combining ability (SCA) of single crosses; and $\mathbf{e} = p \times 1$ vector of residual effects. The $\mathbf{X_0}$, $\mathbf{X_1}$, $\mathbf{Z_1}$, $\mathbf{Z_2}$, and $\mathbf{Z_3}$ incidence matrices related $\mathbf{y}$ to $\boldsymbol{\beta}$, $\boldsymbol{\theta}$, $\mathbf{g_1}$, $\mathbf{g_2}$, and $\mathbf{s}$, respectively.

The covariance matrices for random effects were $V(\mathbf{g_1}) = \mathbf{G_1}V_{\mathrm{GCA1}}$, $V(\mathbf{g_2}) = \mathbf{G_2}V_{\mathrm{GCA2}}$, $V(\mathbf{s}) = \mathbf{S}V_{\mathrm{SCA}}$, and $V(\mathbf{e}) = \mathbf{R}V_{\mathrm{R}}$, where $V_{\mathrm{GCA1}}$ was the variance of GCA effects among BSSS inbreds; $V_{\mathrm{GCA2}}$ was the variance of GCA effects among non-BSSS inbreds; $V_{\mathrm{SCA}}$ was the variance of SCA effects; and $V_{\mathrm{R}}$ was the residual variance. The $i \times j$ element in $\mathbf{G_1}$ was equal to $f_{ij}$, the marker-based estimate of coefficient of coancestry between BSSS inbreds $i$ and $j$. Likewise, the $i' \times j'$ element in $\mathbf{G_2}$ was equal to $f_{i'j'}$ between non-BSSS inbreds $i'$ and $j'$. The element in $\mathbf{S}$ that corresponded to the $(i \times i')$ and $(j \times j')$ pair of single crosses was equal to $f_{ij}f_{i'j'}$. $\mathbf{R}$ was a diagonal matrix with diagonal elements equal to the reciprocal of the number of locations in the corresponding experiment.

Solutions for $\boldsymbol{\beta}$, $\boldsymbol{\theta}$, $\mathbf{g_1}$, $\mathbf{g_2}$, and $\mathbf{s}$ were obtained from the mixed-model equations for single crosses (Henderson 1984; Bernardo 1996a). Restricted maximum likelihood estimates of $V_{\mathrm{GCA1}}$, $V_{\mathrm{GCA2}}$, $V_{\mathrm{SCA}}$, and $V_{\mathrm{R}}$ were obtained as described by Henderson (1984) and Bernardo (1996a). For each trait, the heritability ($h^2$) among single crosses was calculated on an entry-mean basis as $(V_{\mathrm{GCA1}} + V_{\mathrm{GCA2}} + V_{\mathrm{SCA}})/(V_{\mathrm{GCA1}} + V_{\mathrm{GCA2}} + V_{\mathrm{SCA}} + V_{\mathrm{R}}/e_{\mathrm{h}})$, where $e_{\mathrm{h}}$ was the harmonic mean of the number of environments (i.e., location–year combinations) where the single crosses were evaluated. The numbers of environments used to evaluate each single cross corresponded to the diagonal elements of the $\mathbf{Z_3'R^{-1}Z_3}$ diagonal matrix (Bernardo 1996a).

## RR-BLUP for genomewide prediction of single-cross performance

We calculated genomewide marker effects by ridge-regression BLUP (RR-BLUP), which assumes that each marker accounted for the same proportion of genetic variance (Meuwissen et al. 2001). Simulation results have shown only a minimal loss of selection response due to the convenient but incorrect assumption of equal marker variances in RR-BLUP (Bernardo and Yu 2007). Empirical results in maize, barley (*Hordeum vulgare* L.), Arabidopsis, oat (*Avena sativa* L.) and wheat (*Triticum aestivum* L.) (Lorenzana and Bernardo 2009; Guo et al. 2012; Asoro et al. 2011; Heffner et al. 2011a, b) have strongly indicated that RR-BLUP is preferable to Bayesian methods that permit unequal marker variances and epistasis (Meuwissen et al. 2001; Xu 2003; Dekkers 2009).

The linear model for RR-BLUP with $N_M$ markers was similar to Eq. 1:

$$\mathbf{y} = \mathbf{X_0}\boldsymbol{\beta} + \mathbf{X_1}\boldsymbol{\theta} + \mathbf{W_1}\mathbf{m_1} + \mathbf{W_2}\mathbf{m_2} + \mathbf{W_3}\mathbf{m_3} + \mathbf{e}$$

where $\mathbf{y}$, $\mathbf{X_0}$, $\boldsymbol{\beta}$, $\mathbf{X_1}$, $\boldsymbol{\theta}$, and $\mathbf{e}$ were as defined for Eq. 1; $\mathbf{m_1}$ was an $N_M \times 1$ vector of GCA random effects of markers among BSSS inbreds; $\mathbf{m_2}$ was an $N_M \times 1$ vector of GCA random effects of markers among non-BSSS inbreds; $\mathbf{m_3}$ was an $N_M \times 1$ vector of SCA random effects associated with the BSSS and non-BSSS marker alleles at each SNP locus; and $\mathbf{W_1}$, $\mathbf{W_2}$, and $\mathbf{W_3}$ were incidence matrices that related $\mathbf{y}$ to $\mathbf{m_1}$, $\mathbf{m_2}$, and $\mathbf{m_3}$, respectively. In each heterotic group, the GCA random effect at each SNP locus was expressed as half the difference (in a random-effects model) between the means of the two homozygotes at the biallelic SNP locus. The elements of incidence matrices $\mathbf{W_1}$ and $\mathbf{W_2}$ were therefore equal to 1 or $-1$. Given that SCA is a linear × linear interaction, the element of $\mathbf{W_3}$ at a given locus was equal to the product of the elements of $\mathbf{W_1}$ and $\mathbf{W_2}$ at the same locus.

The variances of random marker effects were $V(\mathbf{m_1}) = \mathbf{I}(V_{GCA1}/N_M)$, $V(\mathbf{m_2}) = \mathbf{I}(V_{GCA2}/N_M)$, and $V(\mathbf{m_3}) = \mathbf{I}(V_{SCA}/N_M)$, where $\mathbf{I}$ was an identity matrix and estimates of $V_{GCA1}$, $V_{GCA2}$, and $V_{SCA}$ were those obtained in the BLUP analysis. Solutions for $\boldsymbol{\beta}$, $\boldsymbol{\theta}$, $\mathbf{m_1}$, $\mathbf{m_2}$, and $\mathbf{m_3}$ were obtained from the corresponding mixed-model equations.

## Cross-validation analysis

Tenfold cross-validation was used to compare the accuracy of RR-BLUP versus BLUP. The 479 single crosses were randomly divided into 10 sets with 48 crosses per set (47 in the last set). In each cross-validation analysis, nine of the ten sets formed the training data set to construct genome-wide prediction equations and the remaining set formed the validation data set for single-cross predictions. Estimates of $V_{GCA1}$, $V_{GCA2}$, $V_{SCA}$, and $V_R$ were obtained from the nine sets in each analysis (Henderson 1984; Bernardo 1996a). The performance of the untested hybrids was then predicted as described below, and the correlation between predicted and observed performance (adjusted for fixed effects; see next paragraph) was calculated. This procedure was repeated until each of the ten sets had been assumed untested. Finally, the mean correlation across the ten cross-validation replicates was calculated. All statistical analyses were performed with PROC IML in SAS version 9.1.

The performance of each single cross was first adjusted for fixed effects. Suppose $N_T$ is the number of tested single crosses and $N - N_T = N_U$ is the number of untested single crosses. The adjusted performance of all $N$ single crosses ($\mathbf{y_A}$) was calculated as (Bernardo 1996a).

$$\mathbf{y_A} = (\mathbf{Z_3}'\mathbf{R^{-1}Z_3})^{-1}\mathbf{Z_3}'\mathbf{R^{-1}}(\mathbf{y} - \mathbf{X_0}\boldsymbol{\beta} - \mathbf{X_1}\boldsymbol{\theta})$$

The performance of the $N_T$ tested single crosses ($\mathbf{y_T}$) was obtained as a subset of $\mathbf{y_A}$. The performance of the $N_U$ untested single crosses ($\mathbf{y_U}$) was then predicted from $\mathbf{y_T}$ as (Bernardo 1996a)

$$\mathbf{y_U} = \mathbf{C_{UT}}\mathbf{C_{TT}^{-1}}\mathbf{y_T}$$

where $\mathbf{C_{UT}}$ was an $N_U \times N_T$ matrix of genetic covariances between untested and tested single crosses, and $\mathbf{C_{TT}}$ was an $N_T \times N_T$ matrix of phenotypic covariances among the tested single crosses. Consider the $(i \times i')$ and $(j \times j')$ pair of single crosses, both tested or one of them being an untested single cross. The elements of $\mathbf{C_{UT}}$ as well as the off-diagonal elements of $\mathbf{C_{TT}}$ were calculated as (Stuber and Cockerham 1966)

$$\mathrm{Cov}[(i \times i'), (j \times j')] = f_{ij}\, V_{GCA1} + f_{i'j'}\, V_{GCA2} + f_{ij}f_{i'j'}\, V_{SCA}$$

The three genetic components of single-cross performance in Eq. 1 were therefore expressed in the form of the covariance among single crosses in both $\mathbf{C_{UT}}$ and $\mathbf{C_{TT}}$. The $i$th diagonal element of $\mathbf{C_{TT}}$ was equal to the above equation for the genetic covariance plus $V_R$/($i$th diagonal element of $\mathbf{Z_3}'\mathbf{R^{-1}Z_3}$ matrix).

For RR-BLUP, performance of the untested single crosses ($\mathbf{y_U}$) was predicted as

$$\mathbf{y_U} = \mathbf{U_1}\mathbf{m_1} + \mathbf{U_2}\mathbf{m_2} + \mathbf{U_3}\mathbf{m_3}$$

where $\mathbf{m_1}$, $\mathbf{m_2}$, and $\mathbf{m_3}$ were the genomewide marker GCA and SCA effects among the tested single crosses, and $\mathbf{U_1}$, $\mathbf{U_2}$, and $\mathbf{U_3}$ were incidence matrices that related $\mathbf{y_U}$ to $\mathbf{m_1}$, $\mathbf{m_2}$, and $\mathbf{m_3}$, respectively.

Each BSSS inbred used as a single-cross parent in the test set had been tested in combination with at least one non-BSSS inbred in the estimation set, and vice versa. We also examined the predictive ability of the models when an inbred, used as one of the single-cross parents in the test set, had not been tested in any single-cross combination (Bernardo 1996b). This was accomplished by splitting the BSSS inbreds into ten sets, each set comprising the single crosses that involved the BSSS inbreds in that set. Such partitioning was not done for the non-BSSS inbreds so that only the BSSS parent was assumed untested. Cross validations were then performed as described above.

Prediction accuracies ($r_{MG}$) were expressed as the estimated correlation between the marker-predicted genotypic values and the true genotypic values (Dekkers 2007). The $r_{MG}$ values were calculated as the correlation between marker-predicted values and observed phenotypic values (i.e., adjusted for fixed effects and obtained as the subset of $\mathbf{y_A}$ that corresponded to the $N_U$ untested hybrids), divided by the square root of $h^2$. We used the variance among $r_{MG}$ values across the cross-validation replicates as a measure of the sampling variance of $r_{MG}$ and further used these sampling variance estimates to calculate least significant differences ($P = 0.05$) between the mean $r_{MG}$ with BLUP and with RR-BLUP.

## Genomewide prediction of testcross performance

The genomewide marker effects from RR-BLUP of the single crosses were used to predict testcross performance of doubled haploid lines developed from the same biparental cross and crossed with the same tester. We considered 14 testcross populations undergoing selection in the AgReliant Genetics breeding program. Seven were crosses between BSSS inbreds that were among the 57 BSSS parents of the single crosses. The other seven were crosses between non-BSSS inbreds that were among the 42 non-BSSS parents of the single crosses. The number of progeny in each testcross population ranged from 71 and 292 with a mean of 162. The eight testers were among the 59 BSSS inbreds and 44 non-BSSS inbreds used as parents of the 479 single crosses in this study. All except two testers (N1 and S13) are currently used as parents of AgReliant Genetics

commercial hybrids. Three of the eight testers (N2, S4, and S12) carried transgenes for insect resistance, herbicide tolerance, or both insect resistance and herbicide tolerance. The mean relative maturity of each biparental cross was within 4 days of the relative maturity of the tester used. The parental contribution to inbred progeny was assessed in each population from the SNP data. Because the parents were almost completely inbred, transmission could easily be inferred from the polymorphic markers, and parental contributions were estimated by counting the number of alleles inherited by an inbred from a given parent.

The testcrosses were evaluated in 2007 and 2008 in field trials at 4–10 locations in the US Corn Belt, and the phenotypic data within each testcross population were balanced. Traits measured for testcrosses were the same as those for single crosses. Heritability on an entry-mean basis was estimated from analysis of variance within each testcross population. When $h^2$ was significant, the testcross performance of the individual inbreds was predicted as

$$\mathbf{y_{U1}} = \mathbf{T_1 m_1} + \mathbf{T_3 m_3}$$

and

$$\mathbf{y_{U2}} = \mathbf{T_2 m_2} + \mathbf{T_4 m_3}$$

where $\mathbf{y_{U1}}$ was the predicted testcross performance of inbreds in a BSSS × BSSS cross; $\mathbf{y_{U2}}$ was the predicted testcross performance of inbreds in a non-BSSS × non-BSSS cross; $\mathbf{m_1}$, $\mathbf{m_2}$, and $\mathbf{m_3}$ were the genomewide marker GCA and SCA effects among the tested single crosses; and $\mathbf{T_1}$, $\mathbf{T_2}$, $\mathbf{T_3}$, and $\mathbf{T_4}$ were incidence matrices that related $\mathbf{y_{U1}}$ or $\mathbf{y_{U2}}$ to $\mathbf{m_1}$, $\mathbf{m_2}$, and $\mathbf{m_3}$. As with the $\mathbf{W_3}$ incidence matrix for marker SCA among single crosses, the elements of the $\mathbf{T_3}$ matrix at a given locus were equal to the product of the elements of $\mathbf{T_1}$ and the coded genotypic values (1 or –1) for the tester used. Likewise, the elements of the $\mathbf{T_4}$ matrix at a given locus were equal to the product of the elements of $\mathbf{T_2}$ and the coded genotypic values for the tester used.

The predictions of testcross performance therefore included marker GCA effects for the doubled haploids that were testcrossed and marker SCA effects. Given that comparisons between predicted and observed performance were made only within a biparental population crossed to the same tester, marker GCA effects for the tester were ignored. Predictions were not made if $h^2$ was not significant. Correlations between predicted and observed testcross performance were calculated and were used, along with $h^2$ within each testcross population, in calculating $r_{MG}$.

## Results

The marker-based estimates of coefficient of coancestry ($f_{ij}$) among BSSS inbreds ranged from zero to 0.90, with a mean of 0.49 (Fig. 1). The $f_{ij}$ among non-BSSS inbreds ranged from zero to 0.92, with a mean of 0.28. Heritability among single-crosses (on an entry-mean basis; Bernardo 1996a) was 0.85 for grain yield, 0.98 for grain moisture, 0.83 for stalk lodging, and 0.86 for root lodging. The ratio of $V_{SCA}$ to the total genetic variance among single crosses was 0.18 for grain yield, 0.10 for grain moisture, 0.12 for stalk lodging, and 0.16 for root lodging.

Of the 669 SNP markers, 586 were polymorphic among the BSSS inbreds resulting in a mean marker interval of 3.22 cM on the 1886-cM linkage map used in this study. A total of 598 markers were polymorphic among the non-BSSS inbreds resulting in a mean marker interval of 3.15 cM. In the BSSS group, there were 92 gaps larger than 5 cM and 20 gaps larger than 10 cM. In the non-BSSS group, there were 99 gaps larger than 5 cM and 26 gaps larger than 10 cM.

The LD extended farther in the BSSS population than in the non-BSSS population. The mean $r^2$ between adjacent markers was 0.35 in the BSSS population and 0.26 in the non-BSSS population (Fig. 1). In each heterotic group, more than 40 % of the adjacent markers had an $r^2$ less than 0.10. The percentage of adjacent markers with an $r^2$ greater than 0.20 was 49 % among the BSSS inbreds and 42 % among the non-BSSS inbreds.

When both parents of an untested single cross had available testcross data (denoted by Type 2 in Table 1), the mean prediction accuracy ($r_{MG}$) for BLUP ranged from 0.69 for stalk lodging to 0.90 for grain moisture. The BLUP and RR-BLUP methods did not lead to $r_{MG}$ values that differed significantly.

When one of the BSSS parents of an untested single cross (validation set) had not been tested in any hybrid combination in the training set (denoted by Type 1 in Table 1), the $r_{MG}$ for BLUP decreased for each of the four traits. The least significant differences (LSD) indicated that the $r_{MG}$ estimates were more precise with the Type 2 model than with the Type 1 model. The Type 1 $r_{MG}$ values for BLUP ranged from 0.27 for stalk lodging to 0.75 for grain yield. The corresponding $r_{MG}$ values for RR-BLUP likewise decreased, and none of the $r_{MG}$ values for BLUP was significantly different from the corresponding $r_{MG}$ values for RR-BLUP.

Within each biparental population, the mean parental contribution of the less-represented parental inbred to its progeny ranged from 0.33 to 0.49 (Table 2). The testcross $h^2$ within each population ranged from 0.19 to 0.73 for grain yield (all significantly greater than zero), 0.28 to 0.86 for moisture (all significant), –0.11 to 0.31 for stalk lodging (one of 14 significant), and –0.08 to 0.35 for root lodging (3 of 14 significant). Of the 669 SNP markers that were polymorphic among the single-cross parents, 115–338 (with a mean of 225) were polymorphic within each biparental cross.

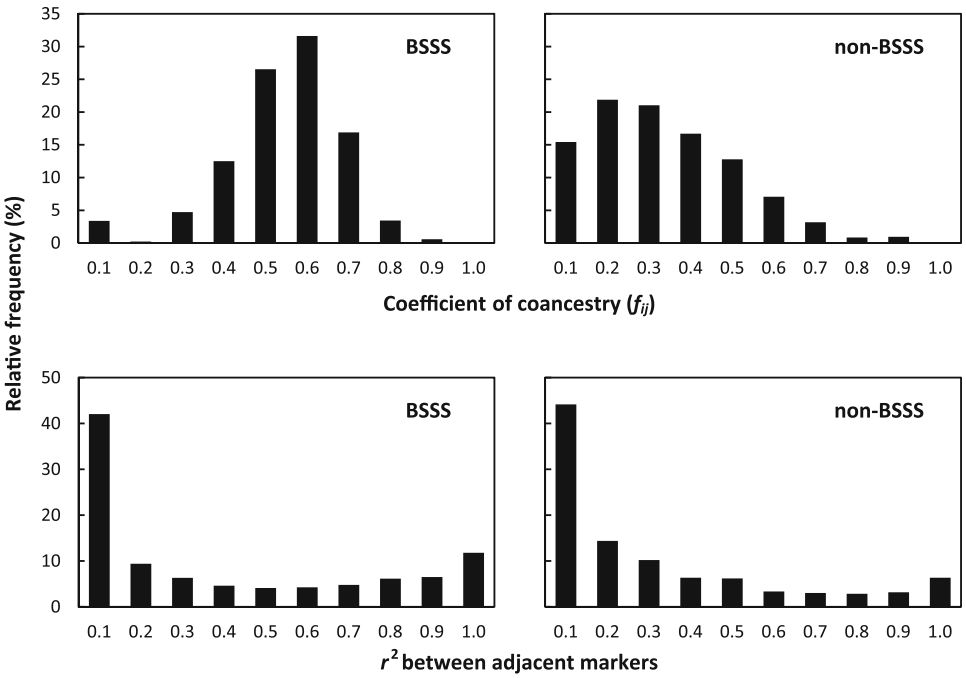


**Fig. 1** Distribution of estimates of marker-based coefficient of coancestry and $r^2$ between adjacent SNP markers among BSSS and non-BSSS inbreds

**Table 1** Cross-validation prediction accuracy ($r_{MG}$) of BLUP and RR-BLUP among maize single crosses

| Model | Grain yield | | Grain moisture | | Stalk lodging | | Root lodging | |
|---|---|---|---|---|---|---|---|---|
| | Type 2[a] | Type 1 | Type 2 | Type 1 | Type 2 | Type 1 | Type 2 | Type 1 |
| BLUP | 0.87 | 0.75 | 0.90 | 0.65 | 0.69 | 0.27 | 0.84 | 0.54 |
| RR-BLUP | 0.87 | 0.73 | 0.88 | 0.64 | 0.66 | 0.31 | 0.81 | 0.53 |
| $LSD^{b}_{0.05}$ | 0.05 | 0.10 | 0.03 | 0.11 | 0.11 | 0.26 | 0.05 | 0.19 |

[a] Type 2 hybrid had both parents included in the model training; Type 1 hybrids had only a single parent included in the model training

[b] Based on the least significant differences (LSD), no significant differences ($P = 0.05$) were found in $r_{MG}$ between BLUP and RR-BLUP for any trait. Each $r_{MG}$ was significantly different from zero

The $r_{MG}$ values within each testcross population were generally low and were often negative, ranging from –0.30 to 0.36 for grain yield and from –0.42 to 0.39 for grain moisture (Table 2). In the one population where stalk lodging had a significant $h^2$ (S7 × S1), the $r_{MG}$ was 0.22. In the three populations where root lodging had a significant $h^2$, none of the $r_{MG}$ values was positive or significant (results not shown). The correlations were generally of the same magnitude between the seven BSSS populations and the seven non-BSSS populations, with the exception of grain moisture. For this trait, the mean $r_{MG}$ was 0.26 for the BSSS populations and –0.21 for the non-BSSS populations.

## Discussion

Our results indicated that RR-BLUP genomewide marker effects estimated from maize single crosses led to high correlations between predicted and observed single-cross performance. However, such high correlations were not superior to those obtained with a previous BLUP approach that did not rely on genomewide marker effects, but instead used random markers to estimate the relatedness among parental inbreds (Bernardo 1994). Our results also indicated that genomewide marker effects estimated from maize single crosses had erratic usefulness for predicting testcross performance within a biparental cross. While our results are contingent upon the population sizes, numbers of markers, and germplasm we used, we believe that the maize data sets in this study were nevertheless typical of the data sets available in maize breeding programs.

High prediction accuracies among single crosses were obtained for all traits studied when the two parents of an untested single cross had testcross data (Type 2 cross validations in Table 1). The high $r_{MG}$ for single-cross performance with BLUP did not leave much room for improvement via RR-BLUP, and the $r_{MG}$ values were equal between RR-BLUP and BLUP for each trait.

**Table 2** Testcross population parameters, parental contributions, testcross heritabilities, and RR-BLUP prediction accuracies within 14 maize biparental populations

| Pedigree | Tester | $N^a$ | $N^b_{Markers}$ | Parental contribution[c] | | Heritability | | | | Prediction accuracy ($r_{MG}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean | Range | Grain yield | Grain moisture | Root lodging | Stalk lodging | Grain yield | Grain moisture |
| S1 × S2 | N1 | 214 | 115 | 0.47 | (0.30, 0.63) | 0.19*[d] | 0.67* | –0.06 | 0.11 | 0.21* | 0.23* |
| S1 × S3 | N1 | 177 | 214 | 0.49 | (0.19, 0.80) | 0.48* | 0.7* | 0.01 | 0.19 | 0.32* | 0.38* |
| S4 × S5 | N1 | 177 | 231 | 0.35 | (0.12, 0.88) | 0.45* | 0.75* | –0.03 | 0.15 | –0.30* | 0.33* |
| S4 × S6 | N2 | 185 | 239 | 0.48 | (0.26, 0.74) | 0.59* | 0.32* | 0.21* | –0.04 | 0.16* | 0.00 |
| S7 × S1 | N2 | 151 | 203 | 0.48 | (0.17, 0.78) | 0.54* | 0.86* | 0.22* | 0.31* | 0.14 | 0.25* |
| S8 × S9 | N2 | 292 | 197 | 0.33 | (0.10, 0.90) | 0.44* | 0.73* | –0.01 | –0.02 | 0.36* | 0.39* |
| S10 × S11 | N3 | 184 | 232 | 0.47 | (0.14, 0.86) | 0.73* | 0.63* | –0.08 | 0.04 | 0.30* | 0.26* |
| N4 × N5 | S10 | 141 | 249 | 0.34 | (0.13, 0.52) | 0.25* | 0.83* | 0.12 | 0.07 | 0.18 | –0.14 |
| N4 × N5 | S12 | 77 | 249 | 0.34 | (0.16, 0.52) | 0.5* | 0.56* | 0.35* | 0.06 | 0.33* | –0.33* |
| N6 × N4 | S7 | 171 | 203 | 0.43 | (0.15, 0.85) | 0.39* | 0.77* | 0.02 | –0.05 | –0.08 | –0.14 |
| N6 × N4 | S12 | 71 | 203 | 0.44 | (0.14, 0.86) | 0.35* | 0.28* | 0.07 | –0.11 | –0.12 | –0.11 |
| N7 × N3 | S4 | 109 | 249 | 0.44 | (0.38, 0.62) | 0.32* | 0.48* | 0.11 | 0.09 | 0.07 | –0.42* |
| N8 × N6 | S13 | 211 | 338 | 0.33 | (0.17, 0.48) | 0.43* | 0.83* | 0.002 | 0.11 | 0.21* | –0.08 |
| N7 × N9 | S4 | 114 | 243 | 0.33 | (0.22, 0.78) | 0.37* | 0.72* | 0.11 | –0.04 | –0.10 | –0.24* |

* Significant at $P = 0.05$

[a] Number of individuals in the biparental population

[b] Number of polymorphic SNPs in the biparental population

[c] Parental contribution of the less-represented parent

Theoretical results (Habier et al. 2007; Goddard 2009; Albrecht et al. 2011) have suggested the equivalency of the RR-BLUP and BLUP approaches, and that markers actually capture relatedness information in RR-BLUP. On the other hand, simulation results (Zhong et al. 2009) have shown that RR-BLUP and BLUP differ in their prediction accuracies under different levels of linkage disequilibrium between markers and QTL. Our unpublished simulation results (R. Bernardo, unpublished data; Supplementary Table 1) showed that in a biparental cross with strong linkage disequilibrium, gains from multiple cycles of selection were 4–22 % higher with RR-BLUP than with BLUP. We interpret this apparent paradox between theoretical results (for the equivalency between RR-BLUP and BLUP) versus simulation results (for the non-equivalency between RR-BLUP and BLUP) to indicate that the contributions of linkage disequilibrium and relatedness are indeed confounded in RR-BLUP but, when linkage disequilibrium between markers and QTL is absent or negligible, RR-BLUP and BLUP are equivalent (Habier et al. 2007). Procedures have been proposed by Habier et al. (2007) and Luan et al. (2012) to estimate the relative contributions of linkage disequilibrium versus relationship information in RR-BLUP. The lack of detailed pedigree information in our study precluded such an analysis.

When one of the parents of a single-cross had not been tested (Type 1 cross validations in Table 1), $r_{MG}$ decreased moderately for grain yield and grain moisture but strongly for root and stalk lodging. As with the Type 2 cross validations, the $r_{MG}$ values for the Type 1 cross validations remained equal between BLUP and RR-BLUP. In BLUP, much of the information exploited in predicting the performance of an $i \times j$ cross is from the testcross performance of $i$ and $j$ themselves (i.e., with other inbreds within the respective heterotic groups; Bernardo 1996b). In the Type 1 cross validations, inbred $i$ itself did not have testcross data in BLUP but the marker alleles in inbred $i$ were represented in RR-BLUP. Our results indicated that the marker effects in RR-BLUP were unable to compensate for the loss of information in BLUP when one parent of the single-cross hybrid was assumed untested. This result needs further investigation.

The square root of heritability ($h$) is defined as the correlation between phenotypic values and the underlying genotypic values. Despite the moderate to high $h^2$ values for grain yield and grain moisture within different biparental crosses (Table 2), the $r_{MG}$ values within biparental crosses were erratic and generally low. While 669 SNPs were polymorphic among the single crosses, only 115–338 SNPs were polymorphic within the biparental crosses. This result indicated that much of the variation among the single

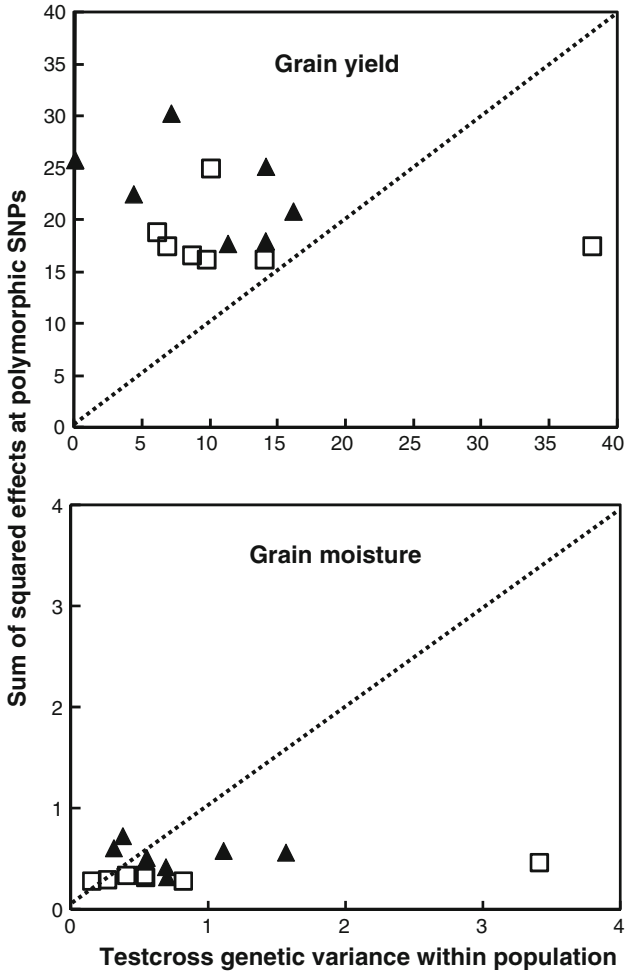


**Fig. 2** Estimates of testcross genetic variance within seven BSSS biparental crosses (*solid triangles*) and seven non-BSSS biparental crosses (*open squares*), versus sum of squared single-cross marker effects at the 115–338 SNP markers that were polymorphic in each cross

crosses was not expressed within a biparental cross, to the extent that prediction equations that work well across diverse single crosses might not work well—and may even lead to negative correlations, as we observed among the non-BSSS biparental crosses—within much smaller pockets of genetic variation represented by biparental crosses. Estimates of testcross variance within the BSSS and non-BSSS biparental populations ($x$ axis in Fig. 2) were indeed smaller than the estimates of GCA variances among single crosses ($V_{GCA1} = 40.3$ among BSSS inbreds and $V_{GCA2} = 40.4$ among non-BSSS inbreds for grain yield; $V_{GCA1} = 3.57$ and $V_{GCA2} = 3.93$ for grain moisture).

Furthermore, genomewide predictions of testcross performance would be accurate only if the genomewide marker effects estimated from single-cross data remain consistent with the marker effects expressed within a biparental cross. One approximate way to gauge such consistency is to compare the testcross variance within an A × B biparental cross with the sum of squares of the marker GCA effects (i.e., estimated from single-cross data) for the subset of markers polymorphic in A × B. For both grain yield and grain moisture, we found a poor correspondence between estimates of within-population testcross variance and the sum of squared effects at polymorphic markers (Fig. 2).

Such poor correspondence may have been caused by genetic drift; by QTL × genetic background interaction; by differences in SNP allele frequencies in the training (single-cross) and prediction (testcross) populations; and by genotype × environment interaction. Differences in SNP allele frequencies would have led to different amounts of information for each homozygote, and the resulting differences in the amount of shrinkage of SNP effects towards the mean may have contributed to differences in marker-effect estimates between the single crosses and testcrosses. Assessing the influence of genotype × environment (particularly genotype × year) interaction on our results is not straightforward. Genotype × year interaction effects were likely confounded with genotypic effects of the 479 single crosses, especially with about 50 % of the single crosses having been evaluated in a single experiment and, therefore, only in a single year (Supplementary Fig. 2). On the other hand, the mean number of locations per experiment was large (25 for grain yield and moisture and 23 for stalk and root lodging; Supplementary Fig. 1) and the exploitation of information from relatives, evaluated in different experiments or years, in BLUP and RR-BLUP (Habier et al. 2007, Goddard 2009) would have lessened the effects of genotype × environment interaction on the predictions. Overall, we surmise that although genotype × environment interactions may have affected the values of $r_{MG}$ per se shown in Table 1, they did not substantially affect our conclusions regarding the usefulness of BLUP versus RR-BLUP for predicting single cross performance. This is because, for predicting single-cross performance, the same training and validation data sets were used in BLUP and in RR-BLUP and both methods were therefore subjected to the same level of confounding of genotypic effects with genotype × environment interaction effects. However, for predicting testcross performance, the environments used to evaluate the training data set (i.e., single crosses) were different from the environments used to evaluate the prediction data set (i.e., biparental crosses). Fewer environments (4–10 locations in 2007 and 2008) were also used to evaluate the testcrosses. We therefore surmise that any reductions in $r_{MG}$ due to genotype × environment interaction in this study were larger for testcross performance than for single cross performance.

We believe that in the context of genomewide prediction, the $r^2$ between adjacent markers is the most

meaningful measure of LD. This is because the $r^2$ between non-adjacent markers is arguably of less consequence as long as the effect of a QTL can be adequately captured by its flanking (i.e., adjacent) markers. Calus et al. (2008) found that genomewide predictions are useful even when the mean $r^2$ between adjacent markers is as low as 0.10, whereas Hayes et al. (2009) recommended an $r^2$ of $\geq$0.20 between adjacent markers. As previously mentioned, the mean $r^2$ between adjacent markers was 0.35 in the BSSS population and 0.26 in the non-BSSS population.

The higher level of LD among the BSSS inbreds than among the non-BSSS inbreds was consistent with the BSSS heterotic group having fewer founder inbreds and, consequently, a narrower genetic base. The same result regarding LD among BSSS inbreds and among non-BSSS inbreds was found by Van Inghelandt et al. (2011). More generally, Van Inglehandt et al. examined the extent of LD with 359 simple sequence repeat and 8,244 SNP markers in 1,537 commercial maize inbred lines belonging to four heterotic groups. The $r^2$ between adjacent markers was lower ($r^2 = 0.28$ among BSSS inbreds) in the Van Inghelandt et al. (2011) study than in our study. On the other hand, the LD in our study was lower than that found by Albrecht et al. (2011), who examined 1,380 dent inbreds (derived from 29 parental inbreds and four single crosses) genotyped with 1,152 SNP markers. Differences in LD among the Van Inghelandt et al. (2011) study, Albrecht et al. (2011) study, and our study can be attributed to the joint effects of differences in the number of inbreds, types of inbred progeny (i.e., recombinant inbreds vs. doubled haploids), genetic diversity, and marker density in each study.

We suggest two possible ways to improve genomewide predictions, particularly for testcross performance. The first is to increase marker density. With recent developments in marker genotyping [Illumina MaizeSNP50 BeadChip (Gupta et al. 2008) and genotyping by sequencing (Glaubitz et al. 2011)] it may be possible to economically increase the number of markers used by many fold. This would allow a stronger LD between markers and QTL and might subsequently allow for more accurate genomewide predictions based on marker-effect estimates from single-cross data. On the other hand, given that factors other than the level of LD (i.e., QTL $\times$ genetic background interaction) may have led to the poor predictions of testcross performance, it is unclear whether a stronger level of LD would have made a substantial difference in our results.

A second way to improve the predictions of testcross performance within biparental crosses is to use multiple, related biparental populations (instead of single crosses) as a training population (Albrecht et al. 2011). In preliminary analysis using a subset of the testcross populations presented here, we were able to predict grain yield with low to moderate accuracy. We calculated RR-BLUP equations from two testcross populations (S1 $\times$ S2 and S7 $\times$ S1) and then applied the prediction equations to three different testcross populations (S8 $\times$ S9, S1 $\times$ S3, and S10 $\times$ S11). The $r_{MG}$ for grain yield was 0.19 for S1 $\times$ S3, 0.27 for S10 $\times$ S11, and 0.62 for S8 $\times$ S9. The data sets in this study were not extensive enough to allow a detailed study of genomewide predictions across multiple populations, and we are currently investigating this method with larger data sets that would allow the identification of factors that contribute to high accuracy of genomewide predictions.

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