

A Novel Mating Design to Optimize Genomic Selection Efficiency for Commercial
Corn Breeding

A Thesis

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Abstract

In many commercial corn (*Zea. mays* L.) breeding programs, lines are selected based only on general combining ability (GCA) during first-year trials. Selection for specific combining ability (SCA) is delayed until later trials, resulting in many unevaluated hybrid combinations. My objective was to determine whether a reciprocal testcross mating design enables simultaneous selection for GCA and SCA, while maintaining the same resources typical in first-year trials. Suppose B1 and B2 are Iowa Stiff Stalk Synthetic (BSSS) lines, whereas N1 and N2 are non-BSSS lines. In a reciprocal testcross design, progeny of B1 × B2 are testcrossed with N1 and N2, and progeny of N1 × N2 are testcrossed with B1 and B2. In 2019, grain yield and moisture of 1,642 hybrids from 10 BSSS and non-BSSS populations were measured at a median of three locations per hybrid across the upper Midwest. In 2020, a validation set consisting of 146 hybrids that were not tested in 2019 were evaluated at a median of five locations per hybrid. The GCA and SCA values were estimated using genomewide prediction with 11,000 SNP markers and the level of dominance was estimated using a subset of these markers. The sizes of training populations were kept constant, and the cross-year predictive ability of the reciprocal testcross design was compared with that of a standard, nonreciprocal design. Including SCA in the models marginally increased predictive abilities for reciprocal designs and the reciprocal designs produced higher predictive abilities than the nonreciprocal designs. The median level of dominance for grain yield was 1.08 indicating complete dominance. The results indicated that the reciprocal testcross mating design combined with

genomic prediction could efficiently enable simultaneous selection for GCA and SCA earlier in a breeding pipeline.

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Abbreviations

RTC – reciprocal test cross

TC – test cross

GCA – general combining ability

SCA – specific combining ability

SNP – single nucleotide polymorphism

BLUP – best linear unbiased prediction

GBLUP – genomic best linear unbiased prediction

RR-BLUP – ridge regression best linear unbiased prediction

GY – grain yield

GM – grain moisture

Chapter 1: Literature Review

Field corn is an international commodity with many uses including food products for human consumption, livestock feed, and ethanol production. In 2021, 93.3 million acres of corn were planted in the United States (USDA-NAAS, 2021). Because of its long history as an important commodity, it has a rich history of breeding efforts aimed at improving the efficiency of its production and understanding the nature of its vigor (Crow, 1998; Hallauer et al., 2010; Nelson, 1993). Many innovations in plant breeding, particularly for hybrid cultivars, were pioneered in corn and those innovations continue to the present day.

Corn is believed to have been domesticated from teosinte in Mexico roughly 9,000 years ago (Matsuoka et al., 2002). Prior to the 20th century corn evolved through the artificial selection of farmers saving seed from the best plants to grow the following year. Such efforts transformed corn from a barely edible grass to a desirable grain crop. Modern corn breeding was pioneered by the observation that a field of open-pollinated corn was composed of a series of very complex hybrids (Shull, 1908). In this seminal work, George Shull documented the deleterious impact of inbreeding and the recovery of vigor when such inbred lines were outcrossed. Several hypotheses have been postulated to describe this phenomenon later called heterosis, but there has yet to be consensus about its cause. This topic is discussed further in Chapter 3.

Following these observations of heterosis in corn, Shull (1909) published his work on “A Pure-Line Method in Corn Breeding,” upon which modern corn breeding is based. With weak parental inbred lines, efficient mass hybrid seed

production remained a hurdle. The usefulness of hybrid vigor was realized a decade after Shull's publication with the invention of double-cross hybrids (Jones, 1918; Jones, 1922). Double-cross hybrids overcame the limitations of weak inbred lines by outcrossing inbred lines to produce more vigorous single-cross hybrids, then again crossing these to other single-cross hybrids to produce the commercial cultivars. The first double-cross hybrids were grown in the 1930s and by the 1950s most of corn grown throughout the country were hybrid cultivars (Crow, 1998).

Since the introduction of hybrid cultivars of corn, researchers have studied how to efficiently breed and select for hybrid performance. *Per se* inbred performance was found to be a poor indicator of hybrid performance (Richey and Mayer, 1925; Smith, 1986) and direct testing of many double cross hybrids is costly and time consuming due to the many combinations of parents that exist and time necessary for seed production. To reduce the extent of field testing for hybrid evaluation, Jenkins and Brunson (1932) showed that the mean performance of an inbred line in several hybrid combinations was reasonably correlated with the average performance of a line crossed to an open-pollinated "tester" cultivar. The method he proposed was that 50% of lines evaluated via these topcrosses could be eliminated without losing any superior inbred lines and the other 50% could be evaluated in different hybrid combinations. Experiments conducted by Jenkins (1934) also showed that the performance of double cross hybrids could be reasonably predicted ($r = 0.61$ for grain yield) from the mean performance of its single cross parents. Furthermore, the contribution of an

inbred line to hybrid performance could be evaluated early in the inbreeding process (Jenkins, 1940).

Originally termed yield prepotency, “the average performance of a line in hybrid combinations” became known as the general combining ability (GCA) of an inbred line (Sprague and Tatum, 1942). In contrast, the authors also defined specific combining ability (SCA) as, “certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines involved.” They compared the relative importance of GCA and SCA in single crosses. In single crosses made among selected lines, SCA was the more important variance parameter, while with unselected lines GCA was more important. They reasoned that lines previously selected based on GCA have more similar GCAs and would primarily differ based on their SCA. Based on these results, Fred Hull (1945) devised a breeding plan to directly select hybrids based on SCA. This scheme involved recurrently selecting and recombining an inbred population based on their combining ability with a single tester which was previously bred for high GCA. In contrast to selection based solely on GCA or on Hull’s SCA method, Comstock et al. (1949) developed a procedure for simultaneous selection of both combining abilities. This breeding procedure was called reciprocal recurrent selection. The authors compared theoretical formulations of the rates and limits of genetic improvement among these three procedures under several scenarios of mode of gene action and allele frequencies. They concluded that in most scenarios reciprocal selection was

theoretically superior to topcross selection for GCA and Hull's method for SCA selection.

Beyond the obvious simplicity and time saving advantages of single-cross hybrids, Cockerham (1961) demonstrated the theoretical increase in genetic gain of single-cross hybrids over double cross hybrids for intra-population improvement. These theoretical results were supported by data showing that single and double-cross hybrids can produce similar yields on average (Stuber et al., 1973). Single crosses became practical with the improvement of inbred lines through recurrent selection and improved management practices (Hallauer et al., 2010). Inbred lines started being used as testers, replacing population cultivars (Russell and Eberhart, 1975). By the 1990s nearly 100% of commercial hybrids in the United States were single crosses.

Hybrid cultivars of relatively narrow genetic base were initially successful over open-pollinated cultivars, but heterosis was further exploited by the delineation of heterotic groups. Throughout the development of hybrid corn in breeding programs, researchers reported on more vigorous hybrids resulting from more diverse crosses (Johnson and Hayes, 1940; Moll et al., 1962; Moll et al., 1965; Wu, 1939). With the passage of the Plant Variety Protection Act in the United States (1970) private companies were incentivized to develop elite inbred lines that maximized heterosis in hybrids. Several groupings have been proposed to assign corn germplasm to heterotic groups (Beckett et al., 2017). In the United States, a common division of heterotic groups is between Iowa Stiff-Stalk Synthetic (BSSS) and non-BSSS (Hallauer et al., 2010; Lu and Bernardo, 2001;

Lu et al., 2009), which can include Iodent, Lancaster, and other sources. Inbred lines belonging to these heterotic groups were derived from open-pollinated or synthetic populations. (Sprague, 1946; Troyer, 1999). Most germplasm of commercial programs in the United States is derived from recombination of a narrow pool of elite inbred lines (Darrah and Zuber, 1986; Lu and Bernardo, 2001; Mikel and Dudley, 2006). As such, lines within a heterotic group tend to have relatively high degree of relatedness.

Single-cross hybrids have been tremendously productive since their introduction in breeding programs. Paired with advancements in biotechnology and crop management, productivity continues to trend upwards. As such, companies competing to produce new and better hybrids each year are interested in evaluating the performance of many single-cross hybrids. However, growing all single-cross combinations of inbreds becomes cost prohibitive with the many inbreds that can be created in a commercial breeding program. Therefore, there is interest in efficient methods to predict single cross performance without making and growing each combination.

Statistical models such as best linear unbiased prediction (BLUP) can be used for such a purpose. Although originally developed for use in dairy cattle breeding (Henderson, 1975), BLUP has become a standard procedure for analysis of plant breeding trials. The simplest definition of BLUP is the estimation of random effects (Robinson, 1991) and it can be used in mixed models to estimate genetic and nongenetic effects in breeding trials. An advantage of BLUP is that it can leverage information from relatives in prediction of genetic effects.

Relationship among genetic materials can be established by pedigree or from genetic marker similarity. Using early marker technologies, BLUP has been successfully used for prediction of single cross performance in a commercial breeding program (Bernardo, 1994; Bernardo, 1995; Bernardo, 1996a; Bernardo, 1996b).

When the relationship matrix in BLUP is calculated using the similarity among genomewide markers, the procedure is called genomic best-linear unbiased prediction (GBLUP). GBLUP is one of a host of procedures used for genomic, or genomewide, prediction. Since the advent of the genomic prediction methodology (Meuwissen et al., 2001), this procedure has been applied to improve the rate of genetic gain in many plant and animal species. Another linear model approach to genomic prediction is ridge-regression BLUP (RR-BLUP). Instead of using covariances among relatives, the effects of genomewide markers are estimated and these effects summed to predict the genetic merit of each individual (Endelman, 2011; Meuwissen et al., 2001). Several linear and nonlinear models have been tested in corn to leverage genomic information for the prediction of genetic variances and effects (Guo et al., 2012; Heslot et al., 2012; Jiang and Reif, 2015; Lorenzana and Bernardo, 2009; Riedelsheimer et al., 2012). These models vary in complexity, types of markers, and statistical assumptions, but generally the differences in predictive ability have been found to be small (Bernardo, 2020b). Genomic prediction methodologies have been successfully applied in the public sector for prediction of testcross (Albrecht et al., 2011; Albrecht et al., 2014; Riedelsheimer et al., 2012; Windhausen et al., 2012)

and single-cross (Dias et al., 2020; Kadam et al., 2016; Massman et al., 2013; Seye et al., 2020; Technow et al., 2014) performance. Single-cross prediction is further discussed in Chapter 2.

Much attention has been given to the topic of training set optimization for genomic prediction. Generally, the factors affecting predictive ability are heritability, size of training set, relatedness between training and test set, and linkage disequilibrium between markers and quantitative trait loci (Bernardo, 2020b). With some theoretical formulations and simulations, training size and heritability were shown to be positively correlated with predictive ability using a GBLUP model, while the number of QTL had no impact (Daetwyler et al., 2008; Daetwyler et al., 2010). Simulations in corn showed that replication had a larger impact on prediction accuracy for lower heritability traits and that prediction accuracy plateaued more rapidly with respect to training set size with higher heritability traits (Lorenz, 2013). Empirical studies on training set optimization within biparental populations were conducted using a large commercial dataset from Monsanto (Brandariz and Bernardo, 2019; Jacobson et al., 2014; Lian et al., 2014). They found that higher predictive abilities were positively correlated not only with larger training sets, but also with more biparental crosses in the training set, higher heritability, and higher LD between adjacent markers (Jacobson et al., 2014; Lian et al., 2014). It was also demonstrated that small highly related training sets constructed for each population produce higher predictive abilities on average than a single large generalized training set for all populations (Brandariz and Bernardo, 2019).

In commercial corn breeding programs, it is often desirable to make predictions across multiple populations so selections can be made across and within families. High predictive abilities can be obtained for cross-population prediction (Zhao et al., 2012). In this study prediction accuracy was 0.58 and 0.90 for grain yield and moisture across six biparental populations. To improve cross-population predictive ability an RR-BLUP model was developed to simultaneously estimate general and population-specific marker effects (Schultz-Streeck et al., 2012). The cross-population predictive abilities for kernel weight and moisture were the same or slightly better with both general and population-specific marker effects than only general marker effects. When trialing intensity per hybrid is limited, genomic selection across populations can improve selection accuracy and stability compared to phenotypic selection (Sleper et al., 2020).

Genomic selection across populations can be effective when properly designed, but predictive ability can vary widely when training set data includes non-target environments and is highly unbalanced across populations (Dias et al., 2020). Unrelated families in training sets can also negatively affect predictive ability (Brauner et al., 2020; Lorenz and Smith, 2015). Intentionally designed trials and training sets are important for genomic prediction to be successful.

In a highly competitive corn breeding market, modern commercial corn breeding programs are interested in maximizing the rate of genetic gain. To do this efficiently, breeding programs tend to be broken into two stages. The first stage to evaluate the GCA of lines and the second stage to evaluate hybrid performance. As such, breeding programs are more interested in estimating

variance affiliated with combining abilities than with genetic variances (Bernardo, 2020a). Chapter 2 deals with a new mating design aimed at optimizing estimation of GCA and SCA in a GBLUP model to predict single-cross performance. Given that GCA and SCA are closely related to the concepts of additive and dominance gene action, Chapter 3 focuses on estimating the level of dominance for grain yield in corn.

Chapter 2: Reciprocal Testcross Mating Design for Single Cross Prediction

Introduction

As commercial corn breeding programs continue to improve the efficiency of their processes, they have increasingly leaned on genomic prediction to evaluate the genetic merit of their germplasm. This technology can be used throughout the breeding process not only for prediction of untested material, but also for more thorough evaluation of material that has been tested in a limited capacity. Genomic prediction in corn breeding can be broadly categorized by three applications: parent prediction, progeny prediction, and hybrid prediction. Parent prediction, which could also be called population prediction, is oriented towards choosing parents to make new breeding crosses and evaluating population parameters of these crosses. Progeny prediction follows as the logical next step in the breeding process. After progenies have been derived from breeding crosses, progeny prediction is used to compare progeny within each population, select the best, and discard the worst prior to field testing. In this category falls testcross prediction because progenies are evaluated for how they generally combine with a series of testers. In contrast, single-cross prediction is the prediction of hybrid performance which involves the evaluation of the GCA of both parents and the SCA. As heterosis has a large impact on corn yields (Shull, 1908), the primary interest of a corn breeding program is the ultimate development of hybrids for commercial release. Therefore, hybrid (i.e. single cross) prediction is of great interest.

A mating design in a plant breeding program is a strategy for how to cross plants and derive progeny. With large populations of random non-inbred progeny, mating designs can be used to estimate how variance is partitioned into genetic and nongenetic components (Hallauer et al., 2010). Such estimations may be useful for informing breeding strategies for novel crops, but for long studied crops like corn estimations are less useful. Rather than estimate variance components to inform breeding strategies, most corn breeding programs are primarily interested in efficient selection strategies, for which mating designs can also be useful.

The testcross design is commonly used in the early stages of a corn breeding pipeline to compare the genetic merit of many inbred lines. Briefly, the testcross design is meant to evaluate the contribution of experimental lines to hybrid performance by using a small number of tester lines to cross to each experimental line (Jenkins and Brunson, 1932). This makes for straightforward comparisons of GCA and efficient seed production. The testcross mating design has been very useful in multi-year corn breeding programs to efficiently test many lines. However, as breeding programs increasingly rely on genomic selection, this design can be optimized for modern applications.

A key component of genomic prediction is the genomic relationship matrix (GRM) which captures the pairwise relatedness among individuals. The GRM has different formulations depending on the component of variance being described and method used for calculation (Aistle and Balding, 2009; VanRaden, 2008). Empirical results have shown that stronger relationships between a

training set and a prediction set results in higher predictive ability (Brandariz and Bernardo, 2019; Jacobson et al., 2014; Lian et al., 2014). Although a testcross design can be very efficient for hybrid seed production and evaluation of general combining ability, it is not designed to maximize relationships for single cross prediction. Generally, hybrids in a testcross design are only related to a single cross through the non-tester parent. In contrast, a factorial design enables relationship through both parents by removing the concept of a tester. This allows more information to be gleaned from each hybrid in the training set.

Consider the untested single crosses among progeny from two families with parents P1-P4 so the pedigree of each cross would be P1/P2//P3/P4. Also consider testcrosses between the progeny of P1/P2 and unrelated tester T1 as well as between the progeny of P3/P4 and unrelated tester T2. In this scenario, full-sib relationship is conferred through the progeny of P1/P2 and P3/P4 and no relationship is conferred through the testers. In a factorial design, a subset of the P1/P2//P3/P4 hybrids could be used to predict the performance of an untested set. Both hybrid parents of each hybrid in such a training set would confer full-sib relationship to the hybrid parents of the untested set. This means that for the purpose of genomic prediction, each hybrid from a factorial design provides twice the information as a testcross hybrid. A factorial design can enable even more of a theoretical advantage when a hybrid relationship matrix is used in the BLUP model. The elements of a hybrid relationship matrix are the product of the elements of the inbred relationship matrices that correspond to the hybrid parents. When one hybrid parent in the training set is unrelated to the untested

hybrid parents, as is the case in the testcross design, the hybrid relationship matrix provides no additional information.

Coancestry in BLUP procedures can be defined either by pedigree or by genetic markers. Using pedigree records to define relatedness in a large commercial dataset, single cross performance was predicted with relatively high accuracy (Bernardo, 1996a). Additionally, including dominance effects in the BLUP models improved predictive ability compared to additive only models (Bernardo, 1996b). Empirical results demonstrating the utility of genomic relationship in BLUP for single-cross performance first came from Bernardo who found high predictive ability ($r=0.8$) for grain yield using RFLP markers and a small panel of phenotyped hybrids (Bernardo, 1994). Using marker-based estimates of coancestry among parents resulted in higher predictive ability than pedigree-based estimated of coancestry, a finding corroborated for predicting testcross performance (Albrecht et al., 2011; Albrecht et al., 2014). Using a similarly small, but much more unbalanced dataset with regard to hybrid combinations and trialing locations, Bernardo (1995) found slightly lower predictive ability ($r=0.75$) than with a balanced dataset using the same number of predictor hybrids. In this study predictive ability was lower ($r=0.49$) when parental data for the untested hybrids were excluded from the training sets.

Most historical data in commercial breeding programs are testcross data and studies have demonstrated the utility of these data for prediction of testcross performance (Albrecht et al., 2011; Albrecht et al., 2014; Massman et al., 2013; Riedelsheimer et al., 2012; Windhausen et al., 2012). Another mating design for

evaluating hybrid performance is a factorial design. This design involves crossing each female parent to each male parent. Empirical results have shown high predictive ability from cross-validation of single-cross performance within a factorial mating design (Kadam et al., 2016; Massman et al., 2013; Technow et al., 2014). Simulations from Seye et al. (2020) showed a benefit to factorial design over the testcross design even when a reciprocal tester was used. However, only a single reciprocal tester was used rather than all parents from the opposite heterotic group and they acknowledged the possibility that the testcross design could be improved if multiple reciprocal testers were used. Empirical results have also suggested that for single-cross prediction, training sets taken from subsets of factorial designs are superior to those from testcross designs (Fritsche-Neto et al., 2018).

A shortcoming of the factorial design is difficulty of hybrid seed production. Many crosses would need to be made by hand in a nursery to produce hybrid seed prior to yield trials. The large number of crosses necessary to enable this design render it infeasible for a large commercial breeding program that tests many thousands of progeny each year. An advantage of the testcross design is that large quantities of seed can be produced in a crossing block which reduces or eliminates the need for mass hand pollinations. To simultaneously leverage the advantages of testcross and factorial design I propose the reciprocal testcross (RTC) design. This design is a special case of the testcross design in which the parents of each inbred family from one heterotic group are used as the testers for the opposite heterotic group. Theoretically, the relationship between a

training set and untested single crosses should be the same for a RTC design and factorial design because for fully inbred lines, parent-offspring has the same coefficient of coancestry as full-sibs (Bernardo, 2020b).

The objective of this chapter is to assess the usefulness of a reciprocal testcross mating design applied in a commercial hybrid corn breeding program. Experiments in this chapter address the hypotheses: 1) There is a significant difference between positive and negative genomic selections of untested single crosses with training data obtained from a reciprocal testcross mating design. 2) By leveraging relationship from both heterotic groups for each cross, predictive ability from a RTC design will be higher than from a TC design. 3) Relative to a TC design, a RTC design increases relative contribution of SCA in a genomic prediction model.

Materials and Methods

Germplasm

Six BSSS inbred lines were crossed to make six biparental families, one of which was a BC₁ family. Five non-BSSS inbred lines were crossed to make three biparental and one three-way family. From each of the 10 families 27-184 (median 82.5) F1-derived doubled haploid (DH) lines were derived resulting in 931 lines. Each DH line was testcrossed to 1-5 (median 2) parent lines from the opposite heterotic group, making a total of 1642 hybrids (Table 1). Ten of the 11 parent lines were used as testers and seven of these testers were traited

versions of the inbred parent lines containing one of four transgenes. All germplasm was owned and contributed by Syngenta Seeds LLC.

Field Experiments

In 2019, each hybrid was grown in a single replication two-row plot (5.9 m by 1.5 m; 0.8 m alleys and 76.2 cm row spacing) at 2-6 (median 3) out of 8 locations across the Midwest. The eight trialing locations were in Hampton, IA, Steward, IL, Spencer, IA, Marshalltown, IA, Eagle Grove, IA, Mineral, IL, Shannon, IL, and Valparaiso, IN. At all the locations each of three check hybrids were grown in 1-19 (median 8) plots throughout the field and all hybrids were randomized across the field. Plots were harvested with a research combine and grain yield and grain moisture were recorded. Grain yield was adjusted to 155 g kg⁻¹ moisture.

Genotyping

Each DH line and tester was genotyped using either an 15K Axiom chip or a Taqman assay. TASSEL 5.0 (Bradbury et al., 2007) was used for genotype filtering and imputation. The LDKNNi function (Money et al., 2015) in TASSEL 5.0 was used for genotype imputation. Function parameters were set to use 30 sites with high linkage disequilibrium (LD) and 25 nearest neighbors and was iterated 14 times. After imputation, sites with missing data (unable to be imputed by LDKNNi) or MAF less than 0.05 were removed. The resulting genotypic dataset had 11,032 SNP markers.

The imputed marker data were used to estimate pairwise coefficients of coancestry (f_{ij}) between lines as in Massman et al. (2013):

$$f_{ij} = \frac{S_{ij} - 0.5(S_{i-} + S_{j-})}{1 - 0.5(S_{i-} + S_{j-})} \quad (2.1)$$

where S_{ij} was the marker similarity between two inbreds and S_{i-} and S_{j-} were the mean marker similarities between an inbred (i or j , respectively) and all inbreds from the opposite heterotic group.

Single Cross Prediction

Single-cross performance was modeled by the BLUP model described in (Bernardo, 2020b):

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{U}\mathbf{g}_1 + \mathbf{W}\mathbf{g}_2 + \mathbf{Z}\mathbf{s} + \mathbf{e} \quad (2.2)$$

With N_{obs} hybrid observations, f fixed effects, N_{g_1} BSSS lines, N_{g_2} non-BSSS lines, and N_s hybrids, \mathbf{y} was a $N_{\text{obs}} \times 1$ vector of plot-level observations, $\boldsymbol{\beta}$ was a $f \times 1$ vector of fixed effects, \mathbf{g}_1 was a $N_{g_1} \times 1$ vector of GCA effects for BSSS lines, \mathbf{g}_2 was a $N_{g_2} \times 1$ vector of GCA effects for non-BSSS lines, \mathbf{s} was a $N_s \times 1$ vector of SCA effects for hybrids, \mathbf{X} was a $N_{\text{obs}} \times f$ incidence matrix relating $\boldsymbol{\beta}$ to \mathbf{y} , \mathbf{U} was a $N_{\text{obs}} \times N_{g_1}$ incidence matrix relating \mathbf{g}_1 to \mathbf{y} , \mathbf{W} was a $N_{\text{obs}} \times N_{g_2}$ incidence matrix relating \mathbf{g}_2 to \mathbf{y} , \mathbf{Z} was a $N_{\text{obs}} \times N_s$ incidence matrix relating \mathbf{s} to \mathbf{y} , and \mathbf{e} was a $N_{\text{obs}} \times 1$ vector of residual effects. The fixed effects ($\boldsymbol{\beta}$) estimated in equation 2.2 were the intercept, locations, checks, and transgenes. The genotype by environment interaction (GxE) was not modeled because each

hybrid was only replicated once within each location. Thus, the GxE variance was confounded with the error variance.

The performance of all 161,700 possible untested single crosses between 700 BSSS DH lines and 231 non-BSSS DH lines was predicted as:

$$\mathbf{y}_U = \mathbf{C}_{UT} \mathbf{C}_{TT}^{-1} \mathbf{y}_T \quad (2.3)$$

where \mathbf{y}_U was a vector of untested single cross performance, \mathbf{C}_{UT} was a $N_U \times N_T$ covariance matrix between N_U untested single crosses and N_T tested single crosses, \mathbf{C}_{TT} was a $N_T \times N_T$ covariance matrix, and \mathbf{y}_T was a $N_T \times 1$ vector of tested single cross performances adjusted for fixed effects (equation). The elements of the hybrid covariance matrices, \mathbf{C}_{UT} and \mathbf{C}_{TT} , were calculated as:

$$C_{ij} = \sigma_{gca_1}^2 f_{ssab} + \sigma_{gca_2}^2 f_{nssyz} + \sigma_{sca}^2 f_{ssab} f_{nssyz} \quad (2.4)$$

where C_{ij} was the covariance between hybrids i and j , f_{ssab} is the coefficient of coancestry between BSSS lines a and b , f_{nssyz} was the coefficient of coancestry between non-BSSS lines y and z , $\sigma_{gca_1}^2$ and $\sigma_{gca_2}^2$ were the estimated variance components for general combining ability of BSSS and non-BSSS lines, and σ_{sca}^2 was the estimated variance component for specific combining ability. These variance components were estimated by an expectation-maximization restricted maximum likelihood (EM-REML) algorithm implemented via the *Gaston* package in R (Dandine-Roulland and Perdry, 2018).

The mixed model equations for single-cross performance was as in Bernardo (2020b):

$$\begin{bmatrix} \beta \\ g_1 \\ g_2 \\ s \end{bmatrix} = \begin{bmatrix} X'X & X'U & X'W & X'Z \\ U'X & U'U + \theta_1 & U'W & U'Z \\ W'X & W'U & W'W + \theta_2 & W'Z \\ Z'X & Z'U & Z'W & Z'Z + \theta_S \end{bmatrix}^{-1} \begin{bmatrix} X'y \\ U'y \\ W'y \\ Z'y \end{bmatrix} \quad (2.5)$$

where

$$\theta_1 = \mathbf{G}_1^{-1}(\sigma_\varepsilon^2/\sigma_{gca_1}^2) \quad (2.6)$$

$$\theta_2 = \mathbf{G}_2^{-1}(\sigma_\varepsilon^2/\sigma_{gca_2}^2) \quad (2.7)$$

$$\theta_S = \mathbf{S}^{-1}(\sigma_\varepsilon^2/\sigma_{sca}^2) \quad (2.8)$$

where \mathbf{G}_1 and \mathbf{G}_2 , were the genomic relationship matrices for BSSS lines and non-BSSS lines, \mathbf{S} was a $N_{\text{hybrid}} \times N_{\text{hybrid}}$ matrix with each element calculated as the product of the elements of \mathbf{G}_1 and \mathbf{G}_2 corresponding the hybrid parents, and σ_ε^2 was the error variance. The elements of \mathbf{G}_1 and \mathbf{G}_2 were pairwise coefficients of coancestry between lines. For illustrative purposes, the coefficients of variance for GCA were alternatively partitioned into the coefficients of variance among lines (σ_{line}^2) and testers (σ_{tester}^2 ; Table 2).

Selection Experiment

Selections were made within each family among the untested single crosses between DH lines based on the predicted ratio of grain yield to grain moisture (GY/GM). A total of 146 selections were made (Table 1). These contained both positive and negative selections within each DH family based on yield/moisture. To align with the goals of a commercial breeding program, more positive selections were made than negative selections. Additionally, about six

tested hybrids from each family were also selected for an additional year of testing, for a total of 61 repeated testcrosses.

In 2020, the 146 previously untested single crosses and 61 repeated testcrosses were grown in a single two-row plot (5.9 m by 1.5 m; 0.8 m alleys and 76.2 cm row spacing) at 2-11 (median 6) out of 15 locations across the Midwest. The 15 trialing locations were Brook, IN, Valparaiso, IN, Malta Bend MO, Clinton, IL, Elwood, IN, Oskaloosa, IA, Markle, IN, Oelwein, IA, Roanoke, IL, Mazon, IL, Sullsburg, WI, Hampton, IA, Steward, IL, Marion, OH, and Eagle Grove, IA. Four locations were in common between years. One check, which was previously grown in 2019, was also grown in 2-8 plots (median 3) at all locations.

Heritability (H^2) in the broad sense was assessed among single crosses on an entry-mean basis as

$$H^2 = \frac{\sigma_{gca_1}^2 + \sigma_{gca_2}^2 + \sigma_{sca}^2}{\sigma_{gca_1}^2 + \sigma_{gca_2}^2 + \sigma_{sca}^2 + \left(\frac{\sigma_{\varepsilon}^2}{n_e}\right)} \quad (2.9)$$

where n_e is the average number of environments per hybrid with environments being defined as a location-year combination.

To evaluate whether previously untested hybrids were successfully selected based on their predicted yield/moisture, t-tests were performed to test for significant differences between the 2020 observed means of positive and negative selections within each family. All positive selections were within the top 5% of single cross predictions within a family and all negative selections were within the bottom 5% of single cross predictions within a family. The *t.test* function

in the stats package in R (R Core Team, 2021) was used with default parameters to conduct two-way tests within each family.

Testcross and Reciprocal Testcross Training Sets

The RTC design is a special case of the TC design in which the parents of the families from one heterotic group are used as the testers for the opposite heterotic group. A balanced RTC design would derive families by mating all combinations of parents within both heterotic groups and use each of those parents as testers for each family from the other heterotic group. For example, with three parents for each heterotic group, say S1-S3 from heterotic group 1 and N1-N3 for heterotic group 2, there would be three families from each heterotic group, S1/S2, S1/S3, S2/S3, N1/N2, N1/N3, and N2/N3. Lines from each S family would be crossed to all N parents and vice versa. With four parents per heterotic group there would be six families per heterotic group and so on. An analogous balanced test cross design would have these same families crossed with unrelated testers by pedigree. A balanced design would also have an equal number of hybrids in each block, with a block being defined as a set of test crosses between one family and one tester.

In order to construct balanced TC and RTC training sets from the 2019 hybrids, each 2020 single cross was categorized by six levels of relationship with hybrids from a balanced TC or RTC design (Table 2). The highest level of relationship is when the test cross family is the same as one of the single cross families and the tester is a parent of the other single cross family. By pedigree, this results in a full-sib relationship on one side of the heterotic group ($f = \frac{1}{2}$) and

a parent-offspring relationship on the other side of the heterotic group ($f = \frac{1}{2}$). The next level is when the test cross family shares one parent with one of the single cross families and the tester is a parent of the other single cross family. This similarly results in a parent-offspring relationship for one heterotic group, but only a half-sib relationship for the other heterotic group ($f = \frac{1}{4}$). The third category has the same family for one heterotic group, but unrelated tester by pedigree. Fourth is a test cross family to unrelated to either single cross family by pedigree, and a tester as a parent of the other single-cross family. The third and fourth levels have the same pedigree relationship to a single cross because full-sib relationship is equal to parent-offspring relationship for inbreds. Fifth, one testcross family shares one parent with a single cross family, and the tester is unrelated by pedigree. Lastly, all parents of the testcross have no pedigree relationship to the single cross.

Training sets were constructed based on theoretical proportions of these six levels of related hybrids that would be derived from balanced testcross or reciprocal testcross designs with three or four parents per heterotic group (Table 2). Some simplifying assumptions were made for the sake of training set construction. Each parent line was considered unrelated by pedigree although by extended pedigree (data not shown) and marker similarity (Table 3; Table 4) these lines are indeed related by pedigree. Also, no distinction was drawn between the parental contribution of three-parent families and biparental families. For example, in the family W/V//Z, W and Z were considered to have the same parental contribution. Consequently, any testcross with tester W or Z would be

considered to have the same parent-offspring relationship to the progeny of W/V//Z. Likewise, the progeny of any family with one parent as W or Z was considered to have the same half-sib relationship to the progeny of W/V//Z. These assumptions were only for the sake of training set construction. Genomic relationship rather than pedigree relationship was used in all BLUP models.

The size of each training set was restricted to eliminate the confounding effect of training set size on predictive ability. This allowed comparisons to be made between predictive abilities from analogous TC and RTC training sets. For example, with a balanced three-parent TC design, 1/3 of the training set has type 3 relationship and 2/3 has type 5 relationship. An analogous three-parent RTC training set has 2/9 type 1, 4/9 type 2, 1/9 type 3, and 2/9 type 5. When predicting the A/B × Y/Z block from this scenario the number of hybrids in each relationship category was 127 type 1, 341 type 2, 165 type 3, and 264 type 5. The training set size was determined as the minimum of the products of the reciprocal of each fraction and the number of hybrids in the respective category: $3 \times 165 = 495$, $3/2 \times 264 = 396$, $9/2 \times 127 = 571.5$, $9/4 \times 341 = 767.25$, $9 \times 165 = 1485$, $9/2 \times 264 = 1188$. The minimum, 396 is the limiting factor so was used as the training set size for this scenario. For each training set, hybrids were randomly sampled from each category until the proportion of hybrids specified by the training set design was fulfilled. When a category was composed of multiple blocks hybrids were evenly sampled among blocks. To account for the variation in predictive ability due to the random sampling, training set construction and prediction was repeated 25 times for each scenario and the average predictive

ability was reported. For the 3-parent designs, the training set sizes ranged from 220 to 649 with an average of 383. For the 4-parent designs, the training set sizes ranged from 265 to 738 with an average of 500.

Assessment of Predictive Ability

Using this two-year dataset, balanced TC and RTC mating designs with three or four parents were compared for their ability to predict untested single crosses according to the mixed-model equations (equation 2.5). Models with all combinations of the GCA and SCA terms in equation 2.5 were evaluated. In each scenario, hybrids from 2019 were used to construct the training sets and single-cross hybrids from 2020 served as the untested prediction sets. Separate training sets were built for each 2020 single cross block, with a block being defined as a set of single crosses between one BSSS family and one non-BSSS family.

Predictive ability was assessed by across-year validation as the correlation between the predicted and observed performance of the 2020 single crosses. The observed performance of each 2020 single cross was the plot-level observation adjusted for fixed effects as in (Massman et al., 2013):

$$\mathbf{y}_T = (\mathbf{Z}'\mathbf{Z})^{-1}\mathbf{Z}'(\mathbf{y} - \mathbf{X}\boldsymbol{\beta}) \quad (2.10)$$

Predictive ability for each scenario was an aggregation of predictions from 19 training sets correlated with BLUEs calculated among 2020 hybrids. One block with three hybrids (A/B × W/V//Z) was excluded from analysis because the

training sets were limited by only having one type 1 hybrid available to construct training sets.

Fisher's z-transformation was used to calculate the 95% confidence intervals around each correlation coefficient (Fisher, 1915).

Multicollinearity between GCA and SCA

Multicollinearity between GCA₁ and SCA was calculated as the correlation between f_{SS} and $f_{SS} \times f_{nSS}$ across all single crosses. Likewise, multicollinearity between GCA₂ and SCA was calculated as the correlation between f_{nSS} and $f_{SS} \times f_{nSS}$ across all single crosses.

Results

Marker-based estimates of coefficients of coancestry varied from 0.36-0.64 for non-BSSS parents (Table 3) and from 0.43-0.72 for BSSS parents (Table 4).

Across the six categories of relatedness between training and prediction set hybrids, the average coefficients of σ_{line}^2 varied from 0.58-0.75, the average coefficients of σ_{tester}^2 varied from 0.51-0.73, and the average coefficients of V_{SCA} varied from 0.29-0.54 (Table 2). The coefficients were consistently highest for type 1 and lowest for type 6. The correlation between the corresponding coefficients of V_{GCA1} and SCA was 0.51 and 0.85 between the coefficients of V_{GCA2} and SCA.

Plot level observations ranged from 4.3 t/ha to 23.3 t/ha with a mean of 13.3 t/ha for grain yield, 130 g/kg to 405 g/kg with a mean of 252 g/kg for grain

moisture and 0.02 to 0.13 with a mean of 0.06 for yield/moisture across both years and approached normal distributions (Figure 1). Correlation between grain yield and moisture was 0.19.

For grain yield, GCA accounted for 29% of the total variance and SCA accounted for 17% in the full cross-year dataset (Table 5). For grain moisture 37% of the total variance was explained by GCA and 20% was explained by SCA. With a median of 3 locations per hybrid, the entry-mean estimates of heritability across years were 0.72 for grain yield, 0.80 for grain moisture, and 0.75 for yield/moisture (Table 5).

Positive and negative selections within each family for yield/moisture showed significant differences in observed single-cross performance. Two-tailed t-tests conducted within each of ten families were significant at the 0.01 level in nine families and significant at the 0.05 level in one family (Table 6).

For all three traits and two design sizes, cross-year predictive abilities were consistently higher on average with the RTC design than with the TC design when the full model ($GCA_1 + GCA_2 + SCA$) was implemented (Table 7). With the TC design, the full model did not improve predictive abilities compared to the GCA only model for grain yield or yield/moisture, and only marginally improved the average predictive ability for grain moisture. In contrast, the full model slightly improved predictive abilities over the GCA only model for all RTC training sets on average, and some changes were statistically significant based on the 95% confidence interval (Table 7).

For grain yield, the highest average predictive ability resulted from the RTC design with the full model for both the three- and four-parent designs (Table 7). For grain moisture and yield/moisture exclusion of GCA_1 from the model with the RTC training set resulted in equal or higher predictive ability compared to the full model with the RTC design or any model with the TC design.

Discussion

The results presented in this chapter suggest that compared to the TC design training sets constructed from a RTC design can have a positive impact on predictive ability for previously untested single crosses (Table 7). Predictive models that included an SCA term improved the predictive ability when RTC designs were used, but not when TC designs were used (Table 7). As expected, based on the relatively high predictive abilities, selection for yield/moisture of previously untested single crosses was successful (Table 6).

Selection Experiments

Positive and negative selections for yield/moisture were significantly different at the 0.05 level in all families indicating the validity of the RTC design for genomic selection training sets (Table 6). The magnitude of significance in these selection experiments was mostly impacted by the group size of the t-tests. This was evident by the two most significant test having at least 11 positive and negatively selected hybrids. Conversely, the test with the least significance had six positive selections and 5 negative selections. As such, these results suggest

that RTC training sets can be effective for large genomic selection experiments undertaken in commercial breeding programs.

Predictive abilities

When the full model was implemented, the predictive abilities of the RTC designs were on average higher than the predictive abilities of the TC designs for all design sizes and traits (Table 7). These predictive abilities were significantly different at the 0.05 level for grain moisture and yield/moisture, but not for grain yield. The TC design did result in a marginally higher, but not statistically significant predictive ability when the GCA only model was used with the 3-parent design. It is possible that the low heritability of grain yield (Table 5) combined with the smaller training set sizes of the three-parent designs relative to the four-parent designs (Table 7) render this result less reliable. Nevertheless, the RTC design resulted in notably higher predictive abilities when SCA is included in the model. These results support the hypothesis that the RTC design enables higher predictive abilities than the TC design due to the higher relatedness between training hybrids and untested single crosses.

Unequal relatedness among untested hybrids and a training set has implications on predictive ability. A simple way of characterizing the relationship between untested hybrids and a training set is by whether or not the hybrid parents exist in the training set. It has been reported that predictive ability from training sets that contain both hybrid parents are superior to those with only one hybrid parent and similarly for those with no hybrid parents (Kadam et al., 2016; Maenhout et al., 2010; Schrag et al., 2010; Technow et al., 2014). Although this

classification is less thorough than the six-tier pedigree relationship defined for an RTC design (Table 2), the RTC design enables inclusion of all hybrid parents in training sets. Of course, it is possible for single crosses with two untested parents to exist within an RTC design if families are only partially tested. A researcher would need to carefully consider resource availability when planning a RTC design. Regardless, with full sibs and reciprocal testers the RTC design benefits from a high degree of relatedness even for single crosses with two untested parents.

Variance Components

The variance components estimated among the hybrid observations in this study indicated that GCA contributed a higher proportion of variance than SCA for all three traits (Table 5). This result aligns with those from similar studies on single cross prediction that also found estimates of GCA variance to be higher than estimates of SCA variance (Kadam et al., 2016; Massman et al., 2013; Technow et al., 2014). On average the estimates of V_{SCA} were higher within RTC training sets than within TC training sets (Table 8). However, inclusion of SCA in the GBLUP models with RTC training sets only slightly increased predictive ability (Table 7). The reason SCA can account for roughly a third of the genetic variance but only slightly increase the predictive ability is multicollinearity between GCA and SCA. Multicollinearity is expected between the coefficients of V_{GCA} and V_{SCA} because V_{SCA} is a product of elements in the \mathbf{G}_1 and \mathbf{G}_2 matrices. The correlation between the coefficients of V_{GCA1} and V_{SCA} was 0.51 and between the coefficients of V_{GCA2} and V_{SCA} was 0.85. Multicollinearity between

GCA and SCA is evident by the different partitions of variance components depending on the model specified (Table 8). For example, when SCA was excluded from the model with the three-parent RTC training set for grain yield, the sum of V_{GCA1} and V_{GCA2} was 0.46 on average. When SCA was included in the model for the same scenario, the sum of V_{GCA1} and V_{GCA2} was only 0.32 and V_{SCA} was 0.18. This suggests that V_{GCA} can account for some, but not all of the variance explained by V_{SCA} .

The training set heritabilities were similar between analogous TC and RTC training sets (Table 8). However, the predictive abilities from RTC training sets were significantly higher than those from the TC training sets (Table 7), likely due to the stronger relationship between the RTC training sets and the untested single crosses. In all scenarios but the 3-parent design for grain yield, the RTC design improved the predictive ability with the GCA only model. This suggests that the improved relatedness of the RTC design improves estimation of both GCA and SCA. Sprague and Tatum gave clear interpretations of the variance parameters estimated for GCA and SCA (Sprague and Tatum, 1942). In summary, the magnitude of V_{GCA} is an indication of the importance of genes that have largely additive effects while V_{SCA} is an indication of the importance of genes that have largely dominance and epistatic effects.

It should be emphasized that the trials in this study were unbalanced with regard to hybrids and environments. Each hybrid was replicated at 2-6 out of 8 locations so the potential genotype-by-environment effects could not be quantified or controlled. In unbalanced designs such as this there is potential for

SCA to be confounded with genotype-by-environment interactions. To illustrate, take for example a factorial design among two BSSS lines, S1 and S2, and two non-BSSS lines, N1 and N2. Single crosses S1/N1 and S2/N1 were grown at location 1 and S1/N2 and S2/N1 were grown at location 2. In this scenario, each single cross observation would need to be simultaneously used to estimate the SCA effects and the GxE effects, so the terms would be inseparable.

Alternatively, if each single cross was grown in both locations the difference between the average performance across locations and the performance at a single location could be used to separate the effects. Likewise, in unbalanced scenarios SCA variance is biased when different sets of locations are used for different hybrids. Therefore, V_{SCA} is likely biased upwards in these experiments due to the inseparable contribution of GxE variance.

Breeding Implications and Future Work

Relatedness between a training set and test set has several implications for the design of a breeding program that uses genomic selection. With greater relatedness, more information can be gleaned from relatives. A simple way to think about how related hybrids are used in genomic prediction is that each relative is a partial replication with more relationship being a larger fraction of replication. A breeding program can use a highly related design like the RTC, to minimize field trials and save resources without sacrificing genetic gain. Furthermore, the RTC design could have implications on the multi-year testing strategies of commercial breeding programs. Often under a testcross design, multiple years are dedicated to selecting lines for good GCA followed by

additional years of selecting hybrids for overall performance (GCA and SCA). The RTC design could be used to replace early-stage testing for GCA and simultaneously allow for early evaluation of single-cross performance. A reasonable proposition could be one year of testing in a balanced RTC design, selection of single crosses, then multiple years of multi-environment trials for single crosses. Given that hybrid performance, not GCA, is of commercial value it seems logical to allocate more resources to hybrid testing and save resources on testcrossing. Also, because combining abilities are context-specific, estimating GCA on a small panel of testers may not represent GCA with the bulk of lines from the opposite heterotic group. The RTC design could provide more robust estimates of GCA compared to the TC design because GCA estimated within the RTC design is more reflective of the broad-based GCA.

The term single cross can be somewhat misleading. Today, most hybrids in competitive seed markets with adequate profit margin are single cross hybrids, but the term in this thesis and in related literature (Kadam et al., 2016; Massman et al., 2013; Technow et al., 2014) is used to refer to those single cross hybrids made between lines derived from the experimental families rather than any hybrids made from a testcross. In the RTC mating design, the quadrant of single cross hybrids is much larger than the other three quadrants (Table 1). The size of quadrant 1 is $N_{NSS} \times N_{SS}$ whereas the size of quadrants 2 and 3 are $N_{NSS} \times N_{Testers_{SS}}$ and $N_{Testers_{NSS}} \times N_{SS}$. One could even consider the smallest, quadrant 4, which is $N_{Testers_{NSS}} \times N_{Testers_{SS}}$. As the number of non-tester lines in any breeding program should far exceed the number of testers, the quadrant of

single crosses should always be the largest. Theoretically, the key benefit of the RTC design is how the smaller quadrants of testcrosses can be used to predict the largest quadrant of single crosses. An interesting extension of this hypothesis is that the fourth quadrant of pairwise testers could be used to predict single cross performance with even fewer hybrids than the current study. The rationale of such a study would be that the parent-offspring relationship between the testers and single cross families is equal to the full-sib relationship among lines within a family. Other designs are possible with different numbers of parents between heterotic groups, not all possible families represented, or not all parents serving as testers. Such variations were not considered in this thesis, but they would have impact on the even distribution of relatedness within the design.

Conclusions

Empirical results in this chapter suggest that a RTC design can enable simultaneous estimates of GCA and SCA and deliver superior predictive ability than a TC design due to increased relatedness between training and prediction sets. Predictive ability is an important metric to evaluate how successful a genomic prediction program will be, but resources required to achieve high predictive abilities is equally important. As discussed, genomic prediction across multiple families can be challenging. Unequal relatedness within a training set can bias predictions toward single crosses that are more related to the training set, even if they are not the highest performers. The results here suggest that the RTC design can be an efficient alternative to the TC design for early-stage testing in commercial corn breeding programs.

Chapter 3: Dominance and the Genetic Basis of Heterosis

Introduction

In diploid organisms, the way alleles interact to confer function is referred to as the mode of gene action. The three basic levels of gene action are additive, dominance, and epistatic. The way pairs of alleles within one locus interact indicates the level of dominance. In the simplest case, no dominance exists when alleles at a locus do not interact. In the absence of dominance, alleles act in a purely additive fashion within a locus and the genotypic value of the heterozygote is equal to the average of the two homozygotes. Conversely, pairs of alleles at a locus are said to act in a purely dominant fashion when one allele completely masks the effect of another allele in a heterozygote. When pairs of alleles at a locus behave somewhere between these two extremes it exhibits partial dominance. Lastly, a heterozygote that has a genotypic value outside the range of either homozygote is said to be overdominant.

Since heterosis in corn was observed and documented by Shull (Shull, 1908), several theories have been postulated for the genetic basis of heterosis. Chief among these theories is the dominance hypothesis. Early observations of dominance patterns for stem thickness and internode length in peas lead to the hypothesis that dominance is responsible for the vigor of F1 hybrids between allelomorphic plants (Keeble and Pellew, 1910). One argument against the dominance hypothesis was that breeders never observed an "instance in which self-fertilization of a corn-plant of maximum vigor has not resulted in a less vigorous progeny" (Shull, 1911). Similarly, some believed varietal size

differences to be fully transmissible by several factors and dismissed the dominance hypothesis (East and Hayes, 1912). They argued that if it were true, they should be able to obtain a vigorous inbred if it had all of the necessary dominant alleles. Having never observed such an inbred they rejected the dominance hypothesis. Alternatively, they postulated “heterozygosis”, later called overdominance, to describe something inherent about the heterozygous state that was superior to the homozygous state. Data were also shown that suggested that hybrids between more dissimilar parents increased heterosis (East and Hayes, 1912). Further argument against the dominance hypothesis was that for other characters regulated by dominant gene action, the F₂ progeny had a patterned asymmetrical segregation of phenotypes (Emerson and East, 1913). Characters of height and yield did not display such skewed segregation thus could not be governed by dominant loci.

Without using any empirical data, Jones countered these arguments using the recent discovery of chromosomes as the units of inheritance (Jones, 1917). He reasoned that if a character is controlled by many loci, linkage would make it virtually impossible for a single inbred to contain all the favorable alleles that could bring about vigor. He also reasoned that a skewed F₂ derived from a vigorous F₁ would not be expected due to the independent assortment of the many loci responsible for heterosis. Collins agreed with the dominance hypothesis but preferred to describe the phenomenon as “suppression of deleterious recessives” rather than “accumulation of dominant growth factors” (Collins, 1921). Seemingly semantic, he argued from an evolutionary perspective

there is nothing inherently advantageous about dominant factors, but there are noticeable disadvantages to many deleterious recessives.

A later argument against the theory of dominance came from data showing that hybrid seed had larger embryos than its parental seeds and this was the cause of heterosis (Ashby, 1930). The counterargument was that increased embryo size was caused by heterosis rather than its cause (East, 1936). East also provided explanation for how dominant gene action could be the reason for increased heterosis between more divergent parents.

A more convincing argument against the dominance hypothesis was from a theoretical formulation suggesting that the expected maximum degree of heterosis should be small; about 5% more than either parent (Crow, 1948). Any vigor beyond that could only be explained by overdominance. Crow suggested, as an alternative to purely dominant or overdominant gene action, that dominance can account for recovery from inbreeding depression to otherwise open-pollinated vigor, and any additional observed vigor must be accounted for by overdominance. Thus, a combination of both dominant and overdominant loci could drive heterosis.

Comstock and Robinson developed a mating design for estimation of the degree of dominance among biparental progenies (Comstock and Robinson, 1948). Using this design and assuming no epistatic variation, Robinson estimated the degree of dominance (d/a) among biparental progenies to be 1.69 for grain yield, thus suggesting overdominance as the mode of gene action (Robinson et al., 1949). However, this study discussed the possibility of how

partial dominance of genes in repulsion-phase linkage could lead to overdominance (i.e., pseudo-overdominance). The conclusions of overdominance or pseudo-overdominance were corroborated by experiments in similarly created backcrosses from random-mated F2 populations (Gardner et al., 1953). The authors also described a method for estimating the degree of linkage-bias in the estimation of d/a . Theoretically, in later generations with more opportunity for recombination, a substantial reduction in the average d/a relative to early generation estimates would suggest repulsion-phase linkage bias in the estimates of d/a in early generations. Such comparisons of early and advanced generation backcrosses supported the linkage bias hypothesis and suggested overdominance not to be the prevalent form of gene action in their populations (Gardner and Lonquist, 1959; Moll et al., 1964).

The next major studies on the genetic basis of heterosis involved the use of molecular markers. This technology enabled marker-level estimates of dominance and additive effects rather than average values across all loci. Using sparse marker data, most significant QTL were found to exhibit overdominance, but could not distinguish between overdominance and pseudo-overdominance (Cockerham and Zeng, 1996; Stuber et al., 1992). Other QTL mapping studies have also reported overdominance and reasoned the likelihood of underlying pseudo-overdominance (Lariepe et al., 2012; Lu et al., 2003).

With conflicting findings over the past century, the degree of dominance for grain yield in corn has yet to be definitively determined. Dominance effects estimated with genomewide marker data have benefited genomic prediction

accuracy (Bernardo, 1996b; Ramstein et al., 2020; Rogers et al., 2021). However, such dense marker data has yet to be used to study the genetic basis of heterosis. Likely, this may be because breeding can be carried out successfully without complete knowledge of the basis of heterosis. Nevertheless, the primary objective of this chapter is to exploit an abundance of genomewide markers and multi-environment trial data from a commercial breeding program to estimate the level of dominance for grain yield in corn using an RRBLUP framework.

Materials and Methods

Empirical Experiment

The germplasm, yield trials, genotype data, and imputation were the same as described in Chapter 2. The marker set for this chapter was filtered to remove any markers that did not have all three genotypes or were redundant with any marker within 50 marker sliding window, resulting in 4805 SNP markers. The average distance between adjacent markers was 0.35 cM.

Genomewide marker effects were modeled by ridge-regression BLUP (RR-BLUP) as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{A}\mathbf{a} + \mathbf{D}\mathbf{d} + \mathbf{e} \quad (3.1)$$

where \mathbf{y} was a $N_{\text{obs}} \times 1$ vector of hybrid observations, \mathbf{X} was a $N_{\text{obs}} \times f$ incidence matrix for fixed effects, $\boldsymbol{\beta}$ was a $f \times 1$ vector of fixed effects for locations, transgenes, and checks, \mathbf{a} and \mathbf{d} were $N_m \times 1$ vectors of additive and dominance effects for each SNP marker, and \mathbf{A} and \mathbf{D} were $N_{\text{obs}} \times N_m$ coded genotypic

matrices relating \mathbf{a} and \mathbf{d} to \mathbf{y} . Markers in \mathbf{A} were coded as 1, 0, or -1 and markers in \mathbf{D} were coded as 0 for homozygotes and 1 for heterozygotes. In \mathbf{X} , each location-year combination was included as a separate factor.

The mixed model equations (MME) used to calculate the additive and dominance marker effects was:

$$\begin{bmatrix} \boldsymbol{\beta} \\ \mathbf{a} \\ \mathbf{d} \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{X} & \mathbf{X}'\mathbf{A} & \mathbf{X}'\mathbf{D} \\ \mathbf{A}'\mathbf{X} & \mathbf{A}'\mathbf{A} + \sigma_{\varepsilon}^2/\sigma_{A_i}^2 & \mathbf{A}'\mathbf{D} \\ \mathbf{D}'\mathbf{X} & \mathbf{D}'\mathbf{A} & \mathbf{D}'\mathbf{D} + \sigma_{\varepsilon}^2/\sigma_{D_i}^2 \end{bmatrix}^{-1} \begin{bmatrix} \mathbf{X}'\mathbf{y} \\ \mathbf{A}'\mathbf{y} \\ \mathbf{D}'\mathbf{y} \end{bmatrix} \quad (3.2)$$

where \mathbf{X} , \mathbf{A} , \mathbf{D} , \mathbf{y} , $\boldsymbol{\beta}$, \mathbf{a} and \mathbf{d} were previously defined. RR-BLUP assumes that the same proportion of genetic variance is accounted for by each marker. The additive and dominance variance components per marker, $\sigma_{A_i}^2$ and $\sigma_{D_i}^2$, and the total residual variance (σ_{ε}^2) were estimated using an EM-REML algorithm implemented by the *Gaston* package in R. For comparison of predictive abilities, the MME was also fit without dominance effects and the additive variance component was re-estimated. The total additive and dominance variance among hybrids was expressed as the product of the per marker variance and the number of markers:

$$\sigma_A^2 = N_m V_{a_i} \quad (3.3)$$

$$\sigma_D^2 = N_m V_{d_i} \quad (3.4)$$

Entry-mean heritability was estimated as:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + (\sigma_\varepsilon^2/n_e)} \quad (3.5)$$

where σ_G^2 was the total genetic variance, σ_ε^2 was the residual variance, and n_e was the average number of environments per hybrid.

The performance of each single cross was adjusted for fixed effects extracted from the MME (equation 3.2) fit to the full cross-year dataset. The adjusted performance of all N single crosses (\mathbf{y}_A) was calculated as (Bernardo, 1996a):

$$\mathbf{y}_A = (\mathbf{Z}'\mathbf{Z})^{-1}\mathbf{Z}'(\mathbf{y} - \mathbf{X}\boldsymbol{\beta}) \quad (3.6)$$

Marker effects were estimated by fitting 2019 data to the additive model or the A+D model. Additive (a) and dominance (d) marker effects were extracted from the solution vector of the MME (equation 3.2) and the level of dominance was estimated at each marker locus as their ratio (d/a). The 146 single crosses tested in 2020 but not tested in 2019 served as the validation data for cross-year prediction. Performance of the untested validation hybrids were predicted as:

$$\mathbf{y}_U = \mathbf{U}_a\mathbf{a} + \mathbf{U}_d\mathbf{d} \quad (3.7)$$

Where \mathbf{y}_U was a $N_U \times 1$ vector of untested single-crosses, \mathbf{U}_a and \mathbf{U}_d were $N_U \times N_m$ coded genotypic matrices relating \mathbf{y}_U to \mathbf{a} and \mathbf{d} .

The cross-year predictive ability (r_{mp}) was expressed as the correlation between \mathbf{y}_U and the subset of \mathbf{y}_A that correspond to the untested hybrids in \mathbf{y}_U .

Simulation Experiment

As RRBLUP is a novel approach for assessing the degree of dominance, a simulation experiment was conducted to evaluate the validity of this approach. Using the AlphaSimR package (Gaynor et al., 2021) two fully inbred diploid parents were simulated with one chromosome 150 cM long and 1000 segregating sites. The average degree of dominance was set to either 0.5, 1, or 1.5 to simulate scenarios of partial, complete, or overdominance, respectively and the variance of the degree of dominance across sites was set to 0.1 for all scenarios. To achieve the same average marker density as the empirical study (0.35 cM), 429 markers were randomly sampled from the available sites. Three different numbers of QTL (20, 80, or 320) were randomly sampled from the non-marker sites.

Three different linkage phases were simulated: repulsion, coupling, and random. Repulsion linkage was simulated by alternating the favorable and unfavorable allele sequentially across the chromosome with one founder having the favorable allele in the even sites and the other founder having the favorable allele in the odd sites. Coupling linkage was simulated by stacking all favorable alleles with one founder and all unfavorable alleles with the other founder. Random linkage phase was simulated by randomly assorting the favorable alleles between the two founders.

A total of 1000 F₂ progeny were derived from the cross between the two founder parents and RRBLUP (equation 3.1) was used to estimate the values of a and d at each marker. The median d/a across all marker loci was calculated and compared to the values set for the degree of dominance. The whole

procedure was iterated 25 times to account for differences due to the sampling of markers and QTL.

Results

In all scenarios of simulated degree of dominance and linkage phase, the degree of dominance estimated via RRBLUP was closest to the true degree of dominance with 20 QTL and furthest from the true degree of dominance with 320 QTL. Random (Figure 2), and to a greater extent repulsion (Figure 3) linkage phase scenarios resulted in the overestimation of the degree of dominance. This overestimation was more pronounced with more QTL and a higher degree of dominance. Under the coupling linkage phase (Figure 4) scenarios more QTL and a higher degree of dominance resulted in lower estimates of the degree of dominance.

For all three traits studied, grain yield, grain moisture and yield/moisture, models including dominance resulted in higher entry-mean heritability and higher cross-year predictive abilities than models with only additive effects (Table 9). The d/a ratio varied from 0.00 to 13803 with a median of 1.08 for grain yield, varied from 0.00 to 1855 with a median of 0.63 for grain moisture, and varied from 0.00 to 672 with a median of 1.03 for yield/moisture (Table 9). The d/a values over 1 suggest overdominance as the mode of gene action and the d/a values between 0 and 1 suggest partial dominance. For grain yield a similar number of markers had an estimated d/a in the partially dominant and overdominant range. For each trait, the level of dominance at marker loci were

evenly distributed across the genome (Figures Figure 5Figure 6Figure 7). The correlation between a and d across SNP loci was -0.11 for grain yield, -0.09 for grain moisture, and -0.15 for yield/moisture. No genomic regions appeared to have noticeably different levels of dominance for any trait.

Discussion

Validity of RRBLUP for Estimation of the Degree of Dominance

As evidenced from the simulation experiment, the RRBLUP estimated values for the degree of dominance varied in their approximation of the true values. When all simulated loci were in repulsion linkage phase RRBLUP overestimated the true degree of dominance. When all simulated loci were in coupling linkage phase RRBLUP estimates were very close to the true degree of dominance. In both linkage phase scenarios, the over or under estimation was more pronounced when more QTL were present on the chromosome. The magnitude of the overestimation in the repulsion linkage phase scenarios increased as the true degree of dominance increased. Conversely, the true degree of dominance had little impact on the estimates under coupling linkage. A random assortment of loci in repulsion and coupling phases resulted in an overestimation of the degree of dominance.

Because of allelic recombination and the large numbers of QTL for quantitative traits, it is unreasonable for a chromosome to contain all coupling phase linkage. Therefore, these results suggest that RRBLUB likely results in an overestimation of the degree of dominance but can provide a good approximation

of the true degree of dominance so long as the number of QTL per chromosome is not exceptionally large (several hundred).

Empirical Estimation of the Degree of Dominance

Similar to previous studies (Bernardo, 1996b; Ramstein et al., 2020; Rogers et al., 2021), modelling dominance improved predictive ability for all three traits. As this result corroborates the results of previous studies, it lends credibility to the model used for the estimation of the degree of dominance. The distribution of the estimated levels of dominance for grain yield and yield/moisture were evenly distributed around 1 (Table 9) which suggests a combination of partially dominant, completely dominant, and overdominant QTL impact grain yield in corn. For comparison, the distribution of these estimates for grain moisture were on average lower than those for grain yield suggesting a greater proportion of QTL for grain moisture are partially dominant. Given the distribution of marker-level d/a estimates, these results indicate a range of gene action impacting grain yield rather than one. Additionally, the possibility of pseudo-overdominance cannot be excluded for those loci with estimates greater than one. The prevailing consensus in the scientific community for the past 60 years has favored the dominance hypothesis (Gardner and Lonquist, 1959; Laripe et al., 2012; Lu et al., 2003; Moll et al., 1964). However, it remains possible that heavy artificial selection for hybrids in corn breeding programs can give rise to modifiers lending to a heterozygote advantage (Fisher, 1928).

Extremely high estimates of the degree of dominance were driven by very low estimates of a rather than very high estimates of d (Figure 8). Such large

estimates of d/a are not likely to be indicative of the true underlying gene action and should be disregarded as outliers. Therefore, the median rather than the mean d/a ratio is a better metric to evaluate the true degree of dominance because it is not skewed by outliers.

To my knowledge, this genomewide RR-BLUP approach has yet to be used to estimate the degree of dominance for any characters. Previous studies have attempted to estimate the degree of dominance in corn using QTL mapping (Cockerham and Zeng, 1996; Lariepe et al., 2012; Lu et al., 2003; Stuber et al., 1992) or the design III mating scheme (Gardner and Lonquist, 1959; Gardner et al., 1953; Moll et al., 1964). Genomewide marker effects is a markedly different approach than the design III mating scheme or QTL mapping for estimation of the degree of dominance. With the design III mating scheme only the average level of dominance can be estimated, but it does not provide any information about the distribution of the level of dominance across individual loci upon which gene action is being inferred. QTL mapping and RR-BLUP are marker-based methodologies that enable estimates of the degree of dominance at individual marker loci. Thus, they can provide information about the average and the distribution of the degree of dominance. The denser marker set used in the current study compared to QTL mapping approaches enables better resolution of linked QTL making the results less prone to linkage bias.

Pseudo-overdominance is apparent overdominance driven by linked QTL with dominant gene action in repulsion phase. This phenomenon has been directly observed in a QTL mapping study. A wide QTL initially thought to be

overdominant (Stuber et al., 1992), when fine-mapped in near-isogenic lines, was indeed two dominant QTL in repulsion phase (Graham et al., 1997). The current study is unable to distinguish between overdominance and pseudo-overdominance. Previous studies using the design III scheme, found that linkage bias to artificially increase the estimated level of dominance (Gardner and Lonquist, 1959; Moll et al., 1964). However, linkage bias was found to have the opposite effect when linked genes are postulated to be in coupling phase (Mareno-Gonzalez et al., 1975). Assuming a majority of linked QTL are in repulsion phase, the high level of dominance observed here could be consequence of low recombination among DH lines. In the simulation experiment, when the degree of dominance was set to 1 and the number of QTL was 20, the average (of the median across markers) estimated degree of dominance was 1.10 with random linkage phase and 1.12 with all repulsion linkage phase. These estimates increased to 1.27 and 1.58 when 80 QTL were simulated, and 1.86 and 3.04 when 320 QTL were simulated. To my knowledge, the number of QTL per chromosome for grain yield in corn is unknown. These simulated results suggest that unless there are many (at least a few hundred) QTL per chromosome and a high degree of repulsion phase linkage the empirical estimated degree of dominance for grain yield, 1.08, is likely driven by a combination of multiple gene actions.

If true overdominant loci do not exist and heterosis is driven by a combination of partially and completely dominant loci, then theoretically after many cycles of true recurrent selection, an inbred should be able to achieve

hybrid levels of vigor. Although inbred lines are routinely grown out in breeding nurseries, they are not routinely grown out in yield trials. To my knowledge, there is little effort for long term selection of inbred per se grain yield in corn. However, historically, much progress has been made for inbred characters in corn. Improvement of inbred yield and stature enabled the transition from double-hybrids to single-cross hybrids (Hallauer et al., 2010) and genetic gain continues in the long-term selection experiment for oil and protein in corn (Rocheford, 2009). If overdominance is a misconception, then selection for inbred performance will eventually approach that of hybrid performance.

Conclusions

The median degree of dominance for grain yield empirically estimated to be 1.08 suggests a range of gene action and pseudo-overdominance could not be excluded for those loci with an estimated d/a greater than one. The simulation experiment suggests this estimation is almost certainly an overestimation, but not so much of an overestimation that the empirical estimate is without merit.

The debate surrounding driver of heterosis goes back over a hundred years. Recent literature seems less concerned with the cause of heterosis and more interested in finding better ways to exploit it. As evidenced by the tremendous genetic gain had in maize and other crops throughout the 20th century and continuing to this day, knowledge of the driver of heterosis, whether dominance, overdominance, epistasis, or some combination therein matters little in practice.

Tables and Figures

Table 1. Summary of Mating Design

			BSSS											
			DH Families					Parents/Testers						
			A/B	C/B/B	A/D	E/B	E/D	E/F	A	B	C	D	E	F
non-BSSS	DH Families	Y/Z	10	9	12		5	8	49	37	50	16	13	49
		W/V//Z	3	8		12	16	3		1			56	
		W/Y	16	7	5	6	6	4	18	23	20		24	
		W/X	8	3	4			1		79	55		54	
	Parents/Testers	W			29	20	76							
		X	37	37	56	22	77	144						
		Y	41	39	22	95	101	159						
		Z		48		95								

Numbers of hybrids mated in an incomplete reciprocal testcross design and trialed in 2019 are shown in quadrants 2 (BSSS parents/testers x non-BSSS DH families) and 3 (BSSS DH families x non-BSSS parents/testers). Numbers of single crosses mated in an incomplete factorial design and trialed in 2020 are shown in quadrant 1 (BSSS DH families x non-BSSS DH families).

Table 2. Coefficients of Variance for TC and RTC designs

Prediction Set Hybrid: P1/P2//P3/P4			Mean (min, max) Coef. of Variance			Training Set Proportions			
Type	Training Set Hybrid		σ_{line}^2	σ_{tester}^2	σ_{sca}^2	TC		RTC	
	Family	Tester				3	4	3	4
1	P1/P2	P3 or P4	0.75 (0.16, 1.00)	0.73 (0.35, 0.91)	0.54 (0.10, 0.91)			2/9	1/12
	P3/P4	P1 or P2							
2	(P1 or P2)/U	P3 or P4	0.65 (0.11, 0.96)	0.72 (0.46, 0.91)	0.47 (0.07, 0.74)			4/9	4/12
	(P3 or P4)/U	P1 or P2							
3	P1/P2	U	0.75 (0.16, 1.00)	0.54 (0.30, 0.89)	0.39 (0.08, 0.89)	1/3	1/6	1/9	1/12
	P3/P4								
4	U/U	P1, P2, P3, or P4	0.59 (0.08, 0.91)	0.70 (0.47, 0.91)	0.41 (0.05, 0.67)				1/12
5	(P1, P2, P3, or P4)/U	U	0.65 (0.09, 0.95)	0.54 (0.34, 0.89)	0.33 (0.04, 0.60)	2/3	4/6	2/9	4/12
6	U/U	U	0.58 (0.08, 0.91)	0.51 (0.34, 0.71)	0.29 (0.04, 0.45)		1/6		1/12

Measured coefficients of variance extracted from \mathbf{G}_1 , \mathbf{G}_2 , and \mathbf{S} for six categories of relatedness between training and prediction set hybrids. Coefficients from \mathbf{G}_1 and \mathbf{G}_2 were separated into coefficients of variance among lines (σ_{line}^2) and testers (σ_{tester}^2) for illustrative purposes. Elements of \mathbf{S} are the coefficients of variance for specific combining ability of hybrids (σ_{sca}^2). Training set proportions for 3 and 4 parent (per heterotic group) testcross (TC) and reciprocal testcross (RTC) designs are for balanced scenarios with regard to family sizes and tester distribution. Relatedness of each generic hybrid in the prediction set with a hybrid in the training set was categorized by pedigree and calculated based on marker similarity.

Table 3. Marker-Based coefficients of coancestry among non-BSSS parents

	X	Z	W
Z	0.64		
W	0.51	0.43	
Y	0.43	0.45	0.36

Table 4. Marker-Based coefficients of coancestry among BSSS parents

	E	B	C	A	F.2	F.1
B	0.69					
C	0.50	0.50				
A	0.68	0.68	0.52			
F.2	0.58	0.55	0.43	0.52		
F.1	0.59	0.56	0.44	0.53	0.95	
D	0.57	0.59	0.43	0.54	0.71	0.72

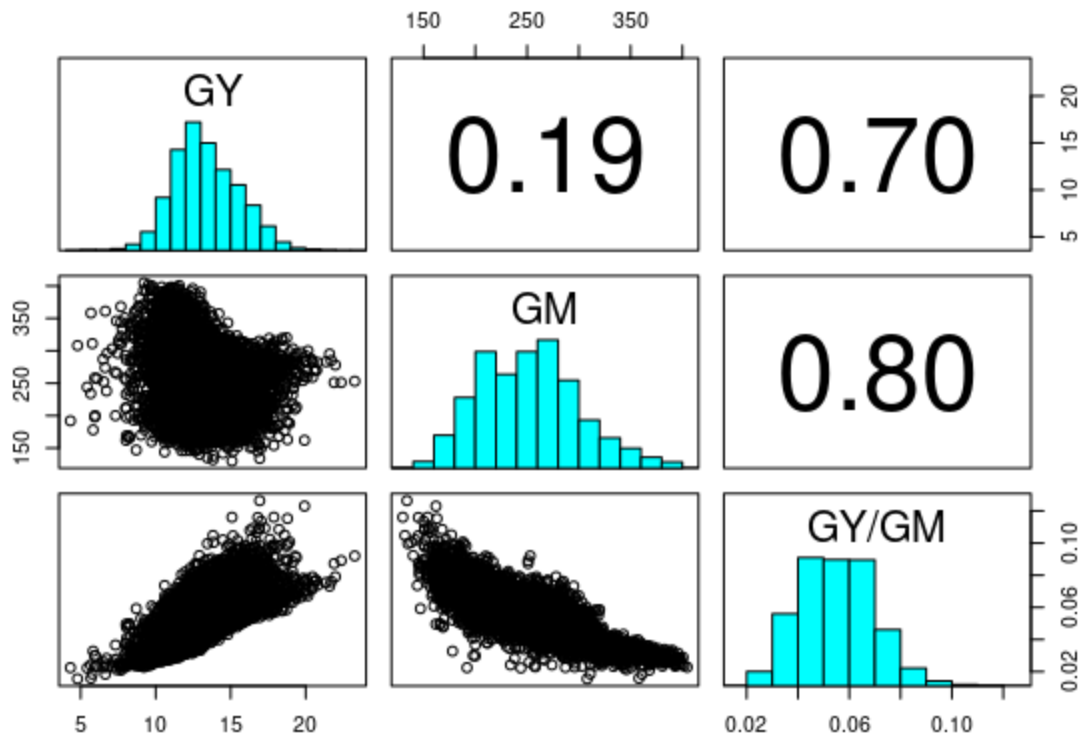


Figure 1. Summary of yield trial observations. 6931 plot-level observations of grain yield (GY; t ha⁻¹), grain moisture (GM; g kg⁻¹) and the ratio of grain yield to grain moisture (GY/GM) among hybrids grown in 2019 and 2020 yield trials.

Table 5. Overall components of variance from yield trials

Trait	Component	proportion of total variance	H ²
GY	$\sigma_{gca_1}^2$	0.15	0.72
	$\sigma_{gca_2}^2$	0.14	
	σ_{sca}^2	0.17	
	σ_{ϵ}^2	0.54	
GM	$\sigma_{gca_1}^2$	0.20	0.80
	$\sigma_{gca_2}^2$	0.17	
	σ_{sca}^2	0.20	
	σ_{ϵ}^2	0.42	
GY/GM	$\sigma_{gca_1}^2$	0.16	0.75
	$\sigma_{gca_2}^2$	0.16	
	σ_{sca}^2	0.17	
	σ_{ϵ}^2	0.50	

Components of variance estimated via an EM-REML procedure for general combining ability among BSSS parents ($\sigma_{gca_1}^2$) and non-BSSS parents ($\sigma_{gca_2}^2$), specific combining ability among hybrids (σ_{sca}^2) and error (σ_{ϵ}^2) for grain yield (GY), grain moisture (GM) and the ratio of grain yield to grain moisture (GY/GM) calculated as a proportion of the total variance among 1790 hybrids grown across two years. Heritability (H²) was calculated as the sum of the genetic components of variance divided by the total variance.

Table 6. Significance test between positive and negative hybrid selections for GY/GM

Family	N Predicted Hybrids	Positive Selections			Negative Selections			P-value from two-sample t-test of measured means
		N	Predicted Mean	Measured Mean	N	Predicted Mean	Measured Mean	
A/B	18018	25	0.054	0.056	5	0.044	0.042	9.90E-03
C/B//B	20328	21	0.055	0.055	5	0.042	0.037	7.09E-03
A/D	14553	12	0.051	0.054	5	0.038	0.038	7.91E-05
E/B	28875	6	0.051	0.052	5	0.040	0.041	4.39E-02
E/D	42504	11	0.047	0.051	14	0.036	0.033	4.52E-08
E/F	37422	11	0.052	0.056	4	0.038	0.037	1.47E-03
Y/Z	42000	30	0.054	0.057	3	0.035	0.029	6.84E-03
W/V//Z	39900	19	0.052	0.052	4	0.036	0.034	8.07E-03
W/Y	18900	11	0.054	0.060	12	0.037	0.035	4.63E-10
W/X	60900	11	0.054	0.050	5	0.038	0.036	7.35E-03

Two-tailed t-tests to test significance of differences of the measured ratio of grain yield to grain moisture (GY/GM) between positive and negative selections. The number of selected hybrids in each t-test group (N) is shown.

Table 7. Predictive abilities of TC and RTC training sets

Np	trait	TC					RTC				
		GCA	GCA + SCA	GCA1+ SCA	GCA2+ SCA	SCA	GCA	GCA + SCA	GCA1+ SCA	GCA2+ SCA	SCA
3	GY	0.30 (0.15,0.44)	0.30 (0.14,0.44)	0.30 (0.15,0.44)	0.29 (0.13,0.43)	0.29 (0.13,0.43)	0.28 (0.12,0.42)	0.33 (0.17,0.46)	0.32 (0.17,0.46)	0.31 (0.15,0.45)	0.30 (0.15,0.44)
	GM	0.67 (0.57,0.75)	0.68 (0.58,0.76)	0.63 (0.52,0.72)	0.66 (0.56,0.74)	0.62 (0.51,0.71)	0.81* (0.74,0.86)	0.82* (0.76,0.87)	0.81* (0.75,0.86)	0.83* (0.77,0.88)	0.82* (0.76,0.87)
	GY/GM	0.57 (0.45,0.67)	0.57 (0.45,0.67)	0.55 (0.42,0.65)	0.56 (0.43,0.66)	0.53 (0.40,0.64)	0.75* (0.67,0.81)	0.77* (0.70,0.83)	0.77* (0.69,0.83)	0.77* (0.70,0.83)	0.77* (0.69,0.83)
4	GY	0.3 (0.14,0.44)	0.29 (0.14,0.43)	0.30 (0.14,0.44)	0.28 (0.12,0.42)	0.28 (0.12,0.42)	0.34 (0.19,0.48)	0.36 (0.21,0.49)	0.36 (0.21,0.50)	0.34 (0.19,0.48)	0.35 (0.20,0.49)
	GM	0.7 (0.61,0.78)	0.71 (0.62,0.78)	0.66 (0.56,0.75)	0.73 (0.64,0.79)	0.69 (0.59,0.77)	0.81* (0.75,0.86)	0.83* (0.77,0.87)	0.82* (0.76,0.87)	0.84* (0.78,0.88)	0.83* (0.77,0.88)
	GY/GM	0.58 (0.46,0.68)	0.57 (0.45,0.67)	0.54 (0.42,0.65)	0.56 (0.44,0.66)	0.53 (0.40,0.64)	0.78* (0.70,0.83)	0.79* (0.72,0.85)	0.78* (0.71,0.84)	0.80* (0.73,0.85)	0.79* (0.72,0.84)

Across-year predictive abilities for training sets from testcross (TC) or reciprocal testcross (RTC) designs with 3 or 4 parents (P) per heterotic group. Traits predicted were grain yield (GY), grain moisture (GM), and the ratio of grain yield to grain moisture (GY/GM). Genomic prediction models with different combinations of terms for general and specific combining abilities were tested (GCA: general combining ability of BSSS and non-BSSS parents, SCA: specific combining ability, GCA₁: general combining ability of BSSS parents, GCA₂: general combining ability of non-BSSS parents). The Fisher's Z 95% confidence interval is shown below the predictive ability. Asterisks indicate the correlation coefficient of the RTC design is significantly different from the correlation coefficient with the same model from TC design at the 0.05 level.

Table 8. Training set variance component summaries

Trait	N _{parents}	Model Terms	Design	$\sigma_{gca_1}^2$	$\sigma_{gca_2}^2$	σ_{sca}^2
GY	3	GCA	RTC	0.26	0.20	
			TC	0.26	0.20	
	GCA+SCA	RTC	0.17	0.15	0.18	
		TC	0.18	0.15	0.16	
	4	GCA	RTC	0.24	0.19	
			TC	0.25	0.20	
GCA+SCA	RTC	0.16	0.14	0.18		
	TC	0.17	0.15	0.16		
GM	3	GCA	RTC	0.36	0.25	
			TC	0.34	0.25	
	GCA+SCA	RTC	0.22	0.17	0.20	
		TC	0.21	0.18	0.18	
	4	GCA	RTC	0.35	0.25	
			TC	0.34	0.24	
GCA+SCA	RTC	0.22	0.18	0.20		
	TC	0.21	0.17	0.18		
GY/GM	3	GCA	RTC	0.28	0.21	
			TC	0.26	0.21	
	GCA+SCA	RTC	0.18	0.16	0.17	
		TC	0.18	0.16	0.15	
	4	GCA	RTC	0.27	0.20	
			TC	0.25	0.21	
GCA+SCA	RTC	0.18	0.15	0.17		
	TC	0.17	0.16	0.15		

Average variance components estimated via an EM-REML procedure in training sets across 19 single cross blocks and 25 iterations for grain yield (GY), grain moisture (GM), and the ratio of grain yield to grain moisture (GY/GM). Training sets were constructed for testcross (tc) and reciprocal testcross (rtc) designs with 3 or 4 parents. Components of variance for general combining ability among BSSS parents ($\sigma_{gca_1}^2$) and non-BSSS parents ($\sigma_{gca_2}^2$) and specific combining ability among hybrids (σ_{sca}^2) are shown for models with terms for general (GCA) and specific (SCA) combining ability.

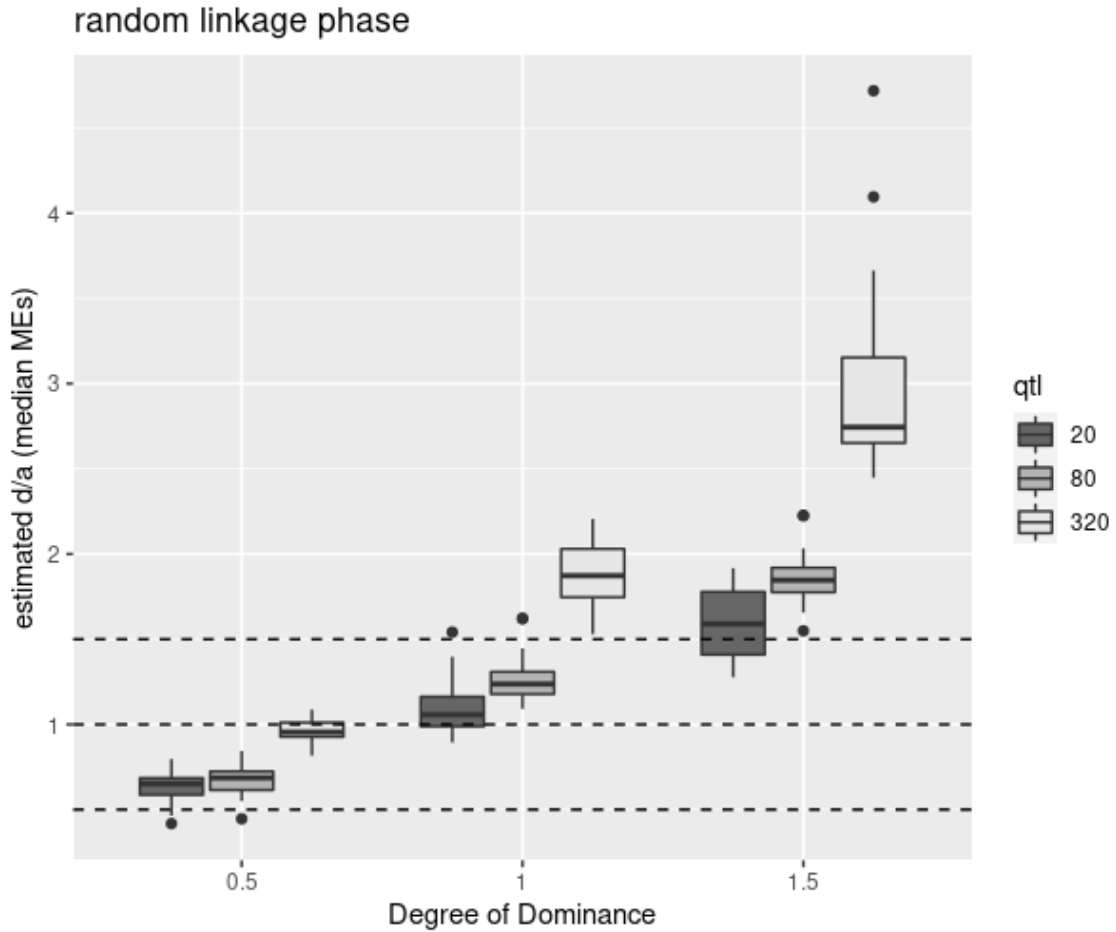


Figure 2. Simulated rrBLUP estimates of the degree of dominance with random linkage phase

Boxplots show the distribution of the median estimates of the degree of dominance (d/a) all markers across 25 iterations. Shadings represent different numbers of simulated quantitative trait loci (qtl) on 1 chromosome. The x-axis is the true degree of dominance for the simulation.

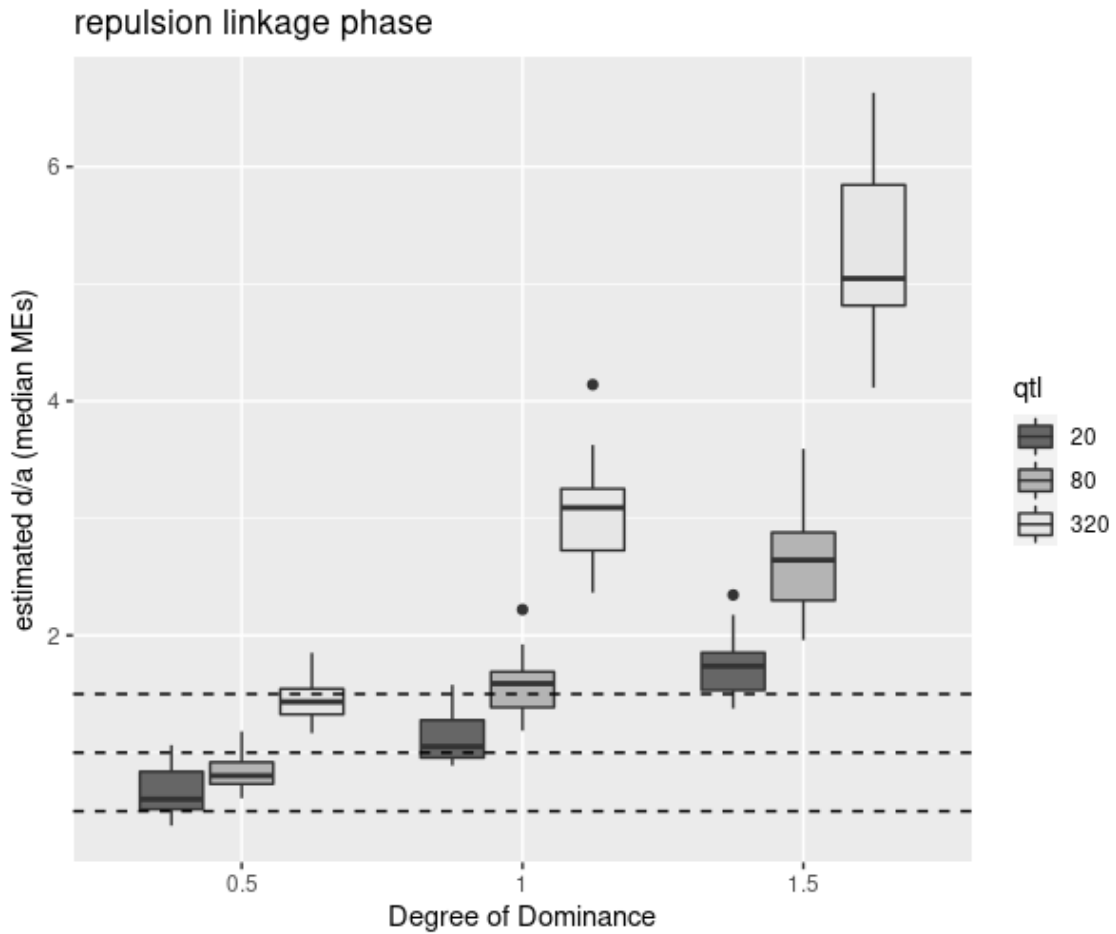


Figure 3. Simulated rrBLUP estimates of the degree of dominance with repulsion linkage phase

Boxplots show the distribution of the median estimates of the degree of dominance (d/a) all markers across 25 iterations. Shadings represent different numbers of simulated quantitative trait loci (qtl) on 1 chromosome. The x-axis is the true degree of dominance for the simulation.

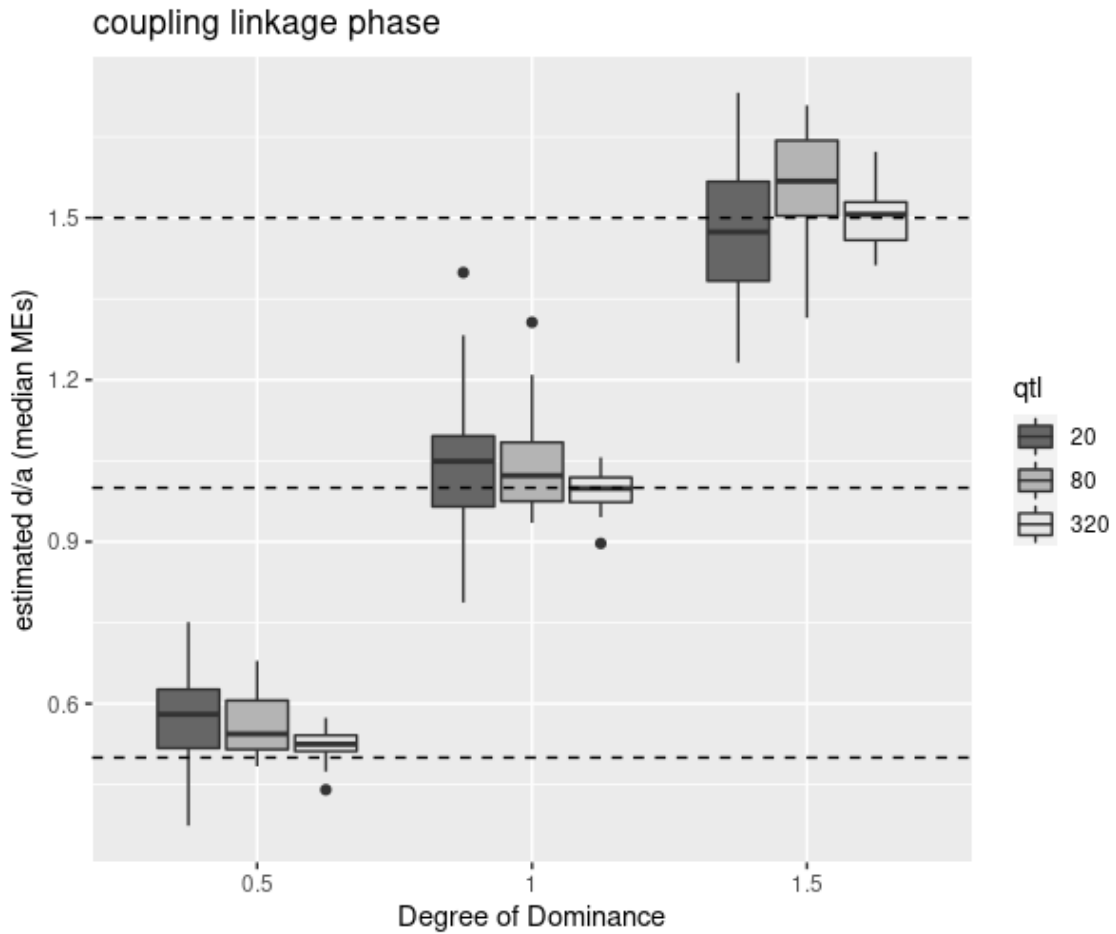


Figure 4. Simulated rrBLUP estimates of the degree of dominance with coupling linkage phase

Boxplots show the distribution of the median estimates of the degree of dominance (d/a) all markers across 25 iterations. Shadings represent different numbers of simulated quantitative trait loci (qtl) on 1 chromosome. The x-axis is the true degree of dominance for the simulation.

Table 9. Summary of predictive ability, variance parameters and dominance effects from yield trials

trait	model	r	Variance Parameters				d/a distribution			
			σ_A^2	σ_D^2	σ_ε^2	H ²	q1	median	mean	q3
GY	A	0.36	0.50		0.50	0.75				
	A+D	0.38	0.30	0.29	0.40	0.82	0.57	1.08	9.4	2.38
GM	A	0.83	0.68		0.32	0.86				
	A+D	0.86	0.43	0.27	0.29	0.88	0.32	0.63	3.32	1.38
GY/GM	A	0.78	0.55		0.45	0.78				
	A+D	0.82	0.33	0.29	0.38	0.83	0.53	1.03	4.22	2.25

Empirical estimates of the degree of dominance (d/a) and additive (σ_A^2), dominance (σ_D^2), and error variance (σ_ε^2) from models with additive (A) or additive and dominance terms (A+D). Traits modeled were grain yield (GY), grain moisture (GM), and the ratio of grain yield to grain moisture (GY/GM). Heritability (H²) was calculated as the ratio of genetic variance to total variance.

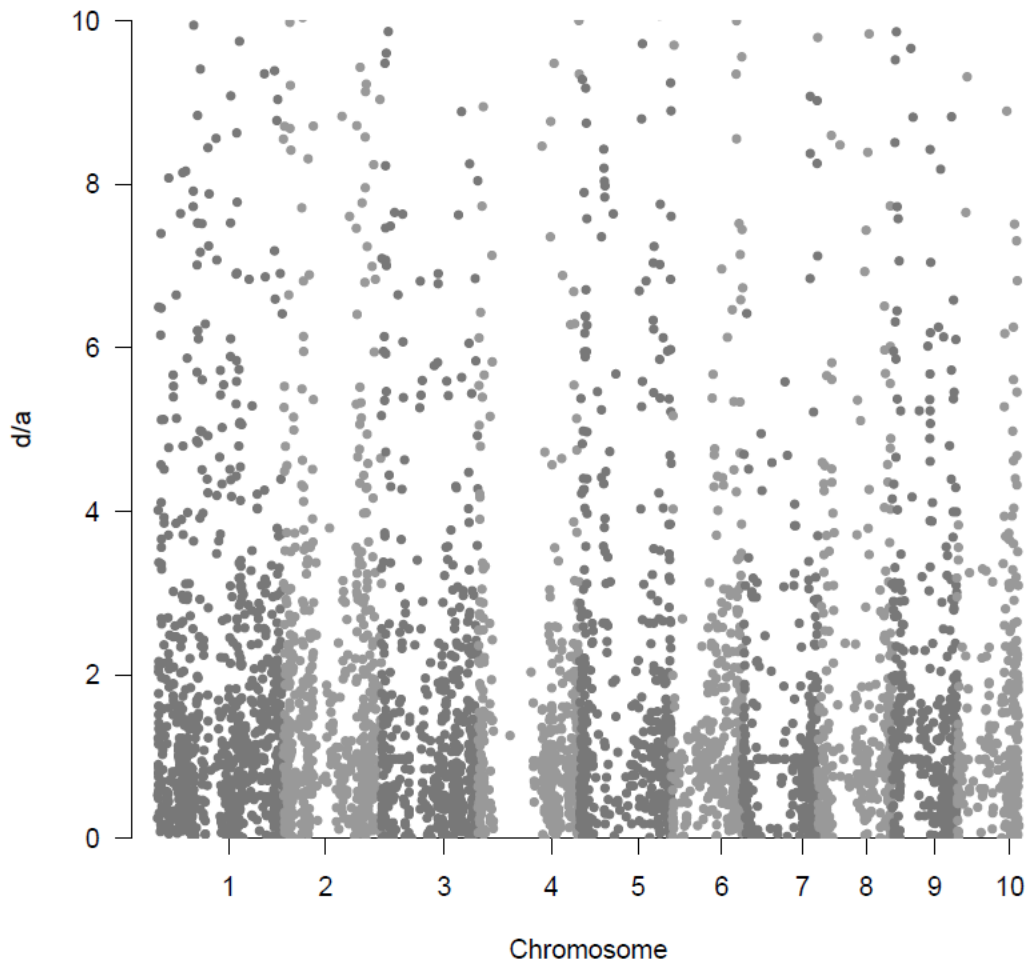


Figure 5. Genomewide d/a marker effects for grain yield

Each dot represents the d/a ratio (y-axis) of a marker plotted by chromosome (x-axis).

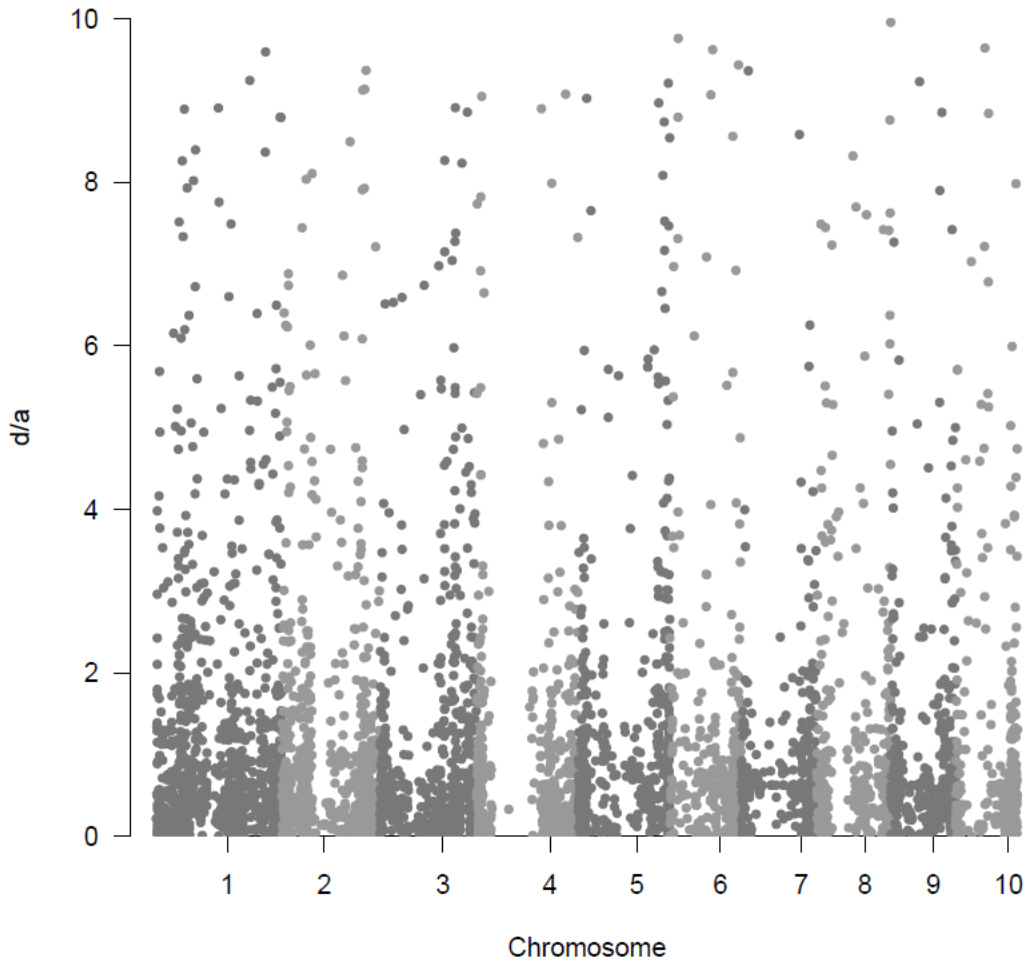


Figure 6. Genomewide d/a marker effects for grain moisture

Each dot represents the d/a ratio (y-axis) of a marker plotted by chromosome (x-axis).

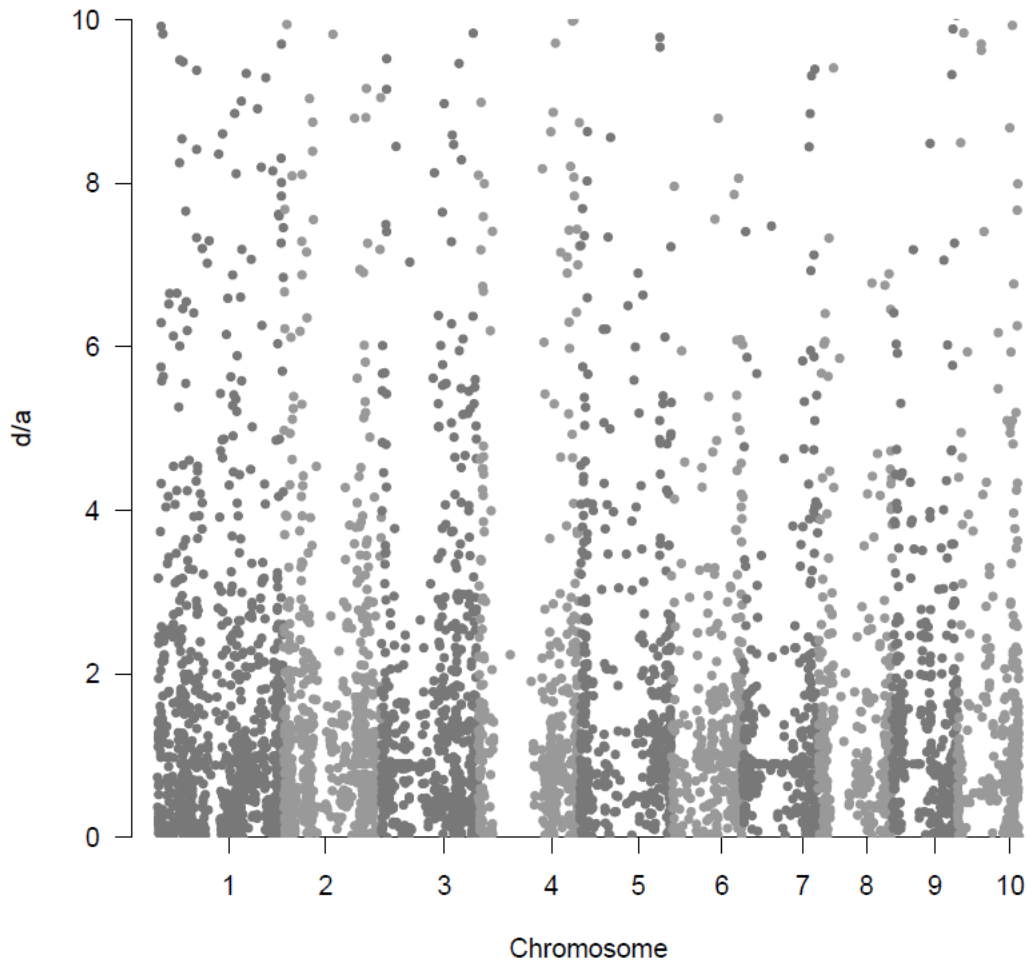


Figure 7. Genomewide d/a marker effects for yield/moisture

Each dot represents the d/a ratio (y-axis) of a marker plotted by chromosome (x-axis).

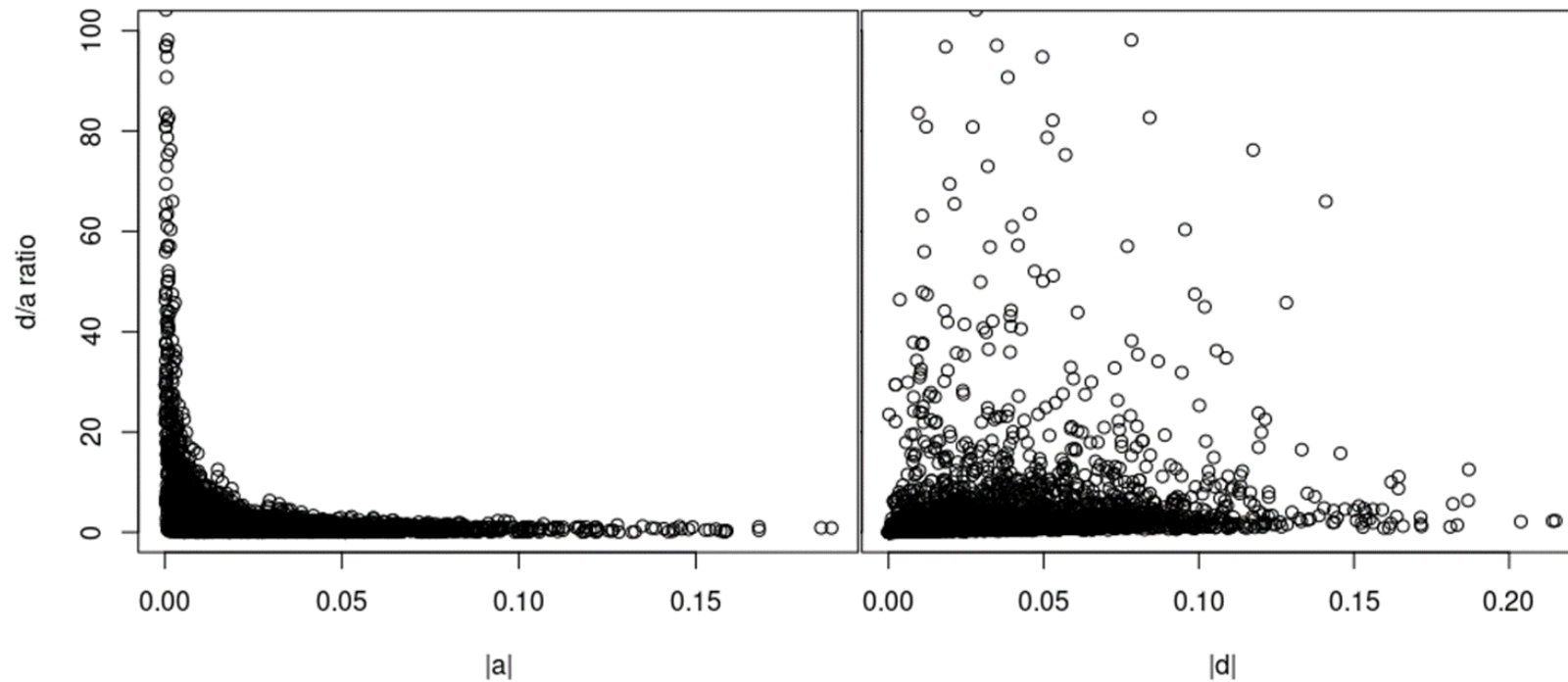


Figure 8. Distribution of a and d vs the degree of dominance for grain yield

The d/a ratio was plotted against the absolute values of a (left pane) and d (right pane). The y-axis was limited to 100 for better visual representation of the relationships, but the max value of d/a is 13803.

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