Incorporation of evolutionary constraint improves genomic prediction of inbred and hybrid phenotype

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ABSTRACT Complementation of deleterious alleles has long been proposed as a major contributor to the hybrid vigor observed in offspring of inbred parents. We test this hypothesis using evolutionary measures of sequence conservation to ask whether incorporating information about putatively deleterious alleles can inform genomic selection (GS) models and improve phenotypic prediction. We measured a number of agronomic traits in both the inbred parents and hybrids of an elite maize partial diallel population. We resequenced the parents of the population, using genomic evolutionary rate profiling (GERP) to identify constrained sites across more than 86 Mb of the genome. We identifed haplotype blocks using an identity-by-decent analysis and scored these blocks on the basis of segregating putatively deleterious variants. Incorporating sequence conservation improves prediction accuracies in a five-fold cross-validation experiment for several traits *per se* as well as heterosis for those traits. These results provide strong empirical support for the simple complementation model of heterosis, and demonstrates the utility of incorporatin functional annotation and its potential usage in phenotypic prediction and plant breeding.

KEYWORDS heterosis; deleterious; genomic selection; diallel; GERP; maize

The phenomenon of heterosis or hybrid vigor has been observed across many species, from yeast (Shapira *et al.* 2014) to plants (Shull 1908) and vertebrates (Gama *et al.* 2013). A number of hypotheses have been put forth to explain the phenomenon, including gene dosage (Birchler *et al.* 2003), overdominance (East 1936; Schwartz 1973; Krieger *et al.* 2010), pseudo-overdomiance (Graham *et al.* 1997; McMullen *et al.* 2009), and epistasis (Minvielle 1987; Schnell and Cockerham 1992). Complementation of recessive deleterious alleles, however, remains the simplest genetic explanation (Charlesworth and Willis 2009), and one that is supported by considerable empirical evidences (Xiao *et al.* 1995; Frascaroli *et al.* 2007; Huang *et al.* 2015).

One of the best studied examples of hybrid vigor is that of maize. Although the benefits of cross-fertilization or hybridization had been observed frequently in the past, it was not until the work of Shull, East, and others that it's significance for agriculture was well appreciated **(CITE)** . Now, hybrid seed makes up the vast majority *in progress, still reading*

Despite the importance of deleterious alleles in contributing to heterosis, they have not been systematically investigated probably because of their low frequencies in the population and mostly exhibiting minor effects. Here, we employed a genomic selection (GS) approach to simultaneously estimate genome-wide deleterious variants in a half diallel population. The diallel population was composed of a set of hybrids, which enabled us to explore different modes of inheritance of the deleterious variants. And the study can be conducted with millions of variants but using relative little sequencing efforts. In our previous study, deleterious SNPs were found to be enriched in a SNP set identified by GWAS (Mezmouk and Ross-Ibarra 2014). The deleterious variants in the study were defined as non-synonymous mutations in the coding regions. Clearly, deleterious variants are

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not limited to coding regions. Here, we expanded the characterization of deleterious variants to genome-wide using genomic evolutionary rate profiling (GERP) (Cooper *et al.* 2005). By incorporating GERP information in GS models, we demonstrated the prediction accuracies were significantly improved not only for some traits *per se*, but aslo for some heterosis transformations (especially for traits exhibiting high levels of hereosis). Further studies indicated that joint effects of deleterious alleles with additive and dominant modes of inheritance may contribute to heterosis.

Materials and Methods

Plant materials and phenotypic data

We selected 12 maize inbred lines, broadly representative of corn belt maize germplasm (Mikel and Dudley 2006), as parents of a partial diallel population. Each parent in a cross was used as both male and female and the resulting seed was bulked (Figure S1). We evaluated the 66 F1 hybrids, 12 inbred parents and two current commercial check hybrids in the field in Urbana, IL over three years (2009-2011) in an incomplete block design with three replicates each year. Plots consisted of four rows, with all observations taken from the inside two rows to minimize effects of shading and maturity differences from adjacent plots. We measured plant height (PHT, in cm), ear height (EHT, in cm), days to 50% silking (DTS), days to 50% pollen shed (DTP), anthesis-silking interval (ASI, in days), grain yield adjusted to 15.5% moisture (adj GY, in bu/A), and test weight (TW, in pounds). Overall mean phenotypic values for each cross can be found at Table S1.

We estimated Best Linear Unbiased Estimates (BLUEs) of the genetic effects in ASReml-R (Gilmour *et al.* 2009) with the following linear model:

$$Y_{ijkl} = \mu + \varsigma_i + \delta_{ij} + \beta_{jk} + \alpha_l + \varsigma_i \cdot \alpha_l + \varepsilon$$

where Y_{ijkl} is the phenotypic value of the l^{th} genotype evaluated in the k^{th} block of the j^{th} replicate within the i^{th} year; μ, the overall mean; ς_i, the fixed effect of the i^{th} year; δ_{ij}, the fixed effect of the j^{th} replicate nested in the i^{th} year; β_{jk}, the random effect of the k^{th} block nested in the j^{th} replicate; α_l, the the fixed genetic effect of the l^{th} individual; $\varsigma_i \cdot \alpha_l$, the interaction effect of the l^{th} individual with the i^{th} year; ε, the model residuals.

We estimated best-parent heterosis (BPH) as:

$$BPH_{min,ij} = \hat{G_{ij}} - min(\hat{G_i}, \hat{G_j})$$

$$BPH_{max,ij} = \hat{G_{ij}} - max(\hat{G_i}, \hat{G_j})$$

where $\hat{G_{ij}}$, $\hat{G_i}$ and $\hat{G_j}$ are the genetic values of the hybrid and its two parents i and j. BPH_{min} was used instead of BPH_{max} for ASI. *what about ear height and DTS?* **Did you mean plant height? We need to discuss about this.** *no plant height should be max, ear height maybe should be min? though it is correlated with plant height... happy to discuss*

Sequencing and Genotyping

We extracted DNA from the 12 inbred lines following Doyle and Doyle (1987) and sheared the DNA on a Covaris (Woburn, Massachusetts) for library preparation. Libraries were prepared using an Illumina paired end libaray protocol with 180 bp fragments. Libraries were then sequenced at Cornell. *Kate Guill. I'll ask.* **text changed.**

We trimmed raw sequence reads for adapter contamination with Scythe (https://github.com/vsbuffalo/scythe) and for quality *Sofiane: what qual score? do we need to say anything about overlapping reads?* and sequence length ($\geq$ 20 nucleotides) with Sickle (https://github.com/najoshi/sickle). We mapped filtered reads to the maize B73 reference genome (AGPv2) with bwa-mem (Li and Durbin 2009), keeping reads with mapping quality (MAPQ) higher than 10 and with a best alignment score higher than the second best one for further analyses. We called single nucleotide polymorphisms (SNPs) using the *mpileup* function from the samtools utilities (Li *et al.* 2009). To deal with known issues with paralogy in maize (Chia *et al.* 2012), SNPs were filtered to be heterozygote in less than 3 inbred lines, have a mean minor allele depth of at least 4, have a mean depth over all individuals lower than 30 and have missing/heterozygote alleles in fewer than 6 inbred lines.

We used the fastIBD method implemented in BEAGLE (Browning and Browning 2009) to impute missing data and identify regions of identity by descent (IBD) between the 12 inbred lines. We then defined haplotype blocks as contiguous regions within which there were no IBD break points across all pairwise comparisons of the parental lines (Figure S2). IBD blocks at least 1 Kb in size were kept for further analysis.

Genomic selection using IBD blocks incorporated with GERP scores

We used genome-wide estimates of evolutionary constraint (GERP Davydov *et al.* 2010) estimated by Rodgers-Melnick *et al.* (2015). Haplotype blocks were weighted by the summed GERP scores of all deleterious (GERP score $>$ 0) SNPs; blocks with no deleterious SNPs were excluded from further analysis. This estimation was calculated under both additive and dominant modes of inheritance using a custom python script available at (https://github.com/yangjl/zmSNPtools). For a particular SNP with a GERP score g, the non-reference homozygote was assigned a value of $2g$, the heterozygote a value of g, and the reference homozygote a value of 0. Under the dominant model, both the heterozygote and the non-reference homozygote were assigned a value of g, with the reference homozygote again assigned a value of 0. To conduct prediction, a 5-fold cross-validation method was used, dividing the diallel population randomly into training (80%) and validation sets (20%) 10 times. The BayesC option from GenSel4 (Habier *et al.* 2011) was used for model training, using 41,000 iterations and removing the first 1,000 as burn-in. After model training, prediction accuracies were obtained by comparing the predicted breeding values with the observed phenotypes in the corresponding validation sets. For comparison, GERP scores were permuted using 50k SNP ($>$ 100Mb) windows which were circularly shuffled 10 times to estimate a null conservation score for each IBD blocks. Cross-validation experiments using the permuted data were conducted on the same training and validation sets.

Results

Genetic values, heritability and heterosis

A partial diallel population was created using 12 maize inbred lines. Two of them are important public inbreds, B73 and Mo17. And the other ten of proprietary inbreds (LH1, LH123HT, LH82, PH207, 4676A, PHG39, PHG47, PHG84, PHJ40, and PHZ51) that have expired from Plant Variety Protection (PVP) and represent much of the lineage of key heterotic germplasm pools used in present-day commercial corn hybrids. From this population,

phenotypic data were collected for seven traits of interest during 2009-2011: anthesis-silking interval (ASI, in days), days to 50% pollen shed (DTP), days to 50% silking (DTS), ear height (EHT, in cm), grain yield adjusted to 15.5% moisture (GY, in bu/A), plant height (PHT, in cm), and test weight (TW, in pounds).

Best linear unbiased estimators (BLUEs) for genotypes of the seven traits were derived from mixed linear models (Table S1). In the models, all fixed effects were significant (Wald test P value < 0.05) for all traits except ASI, for which the effect of replicates within environments were not significant. As shown in Figure 1, BLUE values were normally distributed (normality test P values > 0.05). Broad sense heritability for these traits ranged from 0.65 for ASI to 0.95 for PHT. Using the parental phenotypic data, we then estimated best-parent heterosis (BPH) for each trait. Because the selected inbred lines are commercially relevant and fairly elite in performance, hybrids in this population exhibit relatively low hybrid vigor (overall mean percent BPH = 0.3% $\pm$ 0.4%) for most traits except GY (mean percent BPH = 95% $\pm$ 16%, Figure S3). Finally, general and specific combining ability (GCA and SCA) were estimated following (Falconer and Mackay 1996). GCA and SCA varied among traits (Table S2), but B73, PHG47 and PHG39 showed the greatest GCA for grain yield.

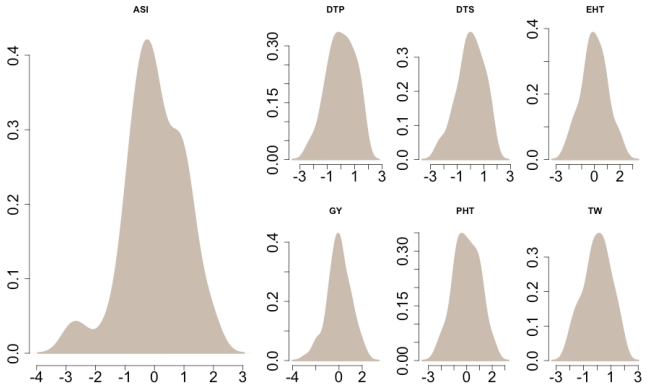

Figure 1 Density plots of the BLUE values for the seven phenotypic traits. On the x-axis, the phenotypic values were normalized.

Sequence variation and evolutionary constraint

All twelve inbreds were sequenced to an average depth of ~10X, resulting in a filtered set of 13.8 million SNPs. We estimated the allelic error rate using three independent data sets: for all individuals using 41,292 overlapping SNPs on the maize SNP50 bead chip (van Heerwaarden *et al.* 2012); for all individuals using 180,313 overlapping SNPs identified through genotyping by sequencing (GBS) (Romay *et al.* 2013); and for B73 and Mo17 using the 10,426,715 SNP from the HapMap2 project (Chia *et al.* 2012). Compared to corresponding SNPs identified by previous studies, a concordance rate of 99.1% was observed. *Sofiane: can we separate those numbers out by study? or just report for one study and mention that similar rates were seen in other studies? either way it would be nice to know what rate went with what data. also is concordance mean identical genotype? do we have minor allele rate (which is a bit more informative)? if not, skip it.*

More than 86 million bp of the genome were annotated as conserved, with GERP scores > 0. Nonetheless, 506,898 of these sites were found to segregate among the 12 inbred parents of our diallel (Figure 2A and S4). The minor allele frequency of SNPs at conserved sites was negatively correlated with GERP score (Figure 2B; P value < 0.05, $r = -0.8$), consistent with the idea that variants at sites with more positive GERP scores are more deleterious and more strongly impacted by purifying selection.

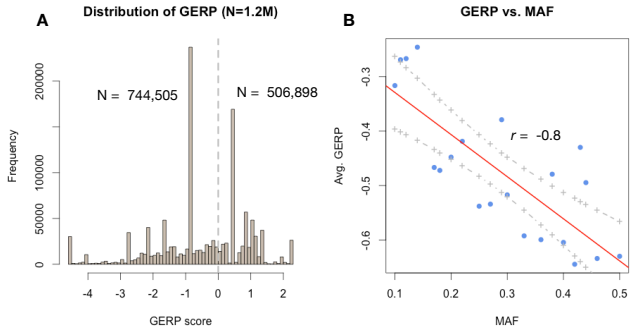

Figure 2 Distribution of GERP scores and relationship between GERP scores and MAFs. **(A)** Histogram of GERP scores at ~1.3 million SNPs. **(B)** Plot of average GERP scores in bins (bin size = 0.01) of minor allele frequency (MAF). Red and grey lines define the regression and its 95% confidence interval.

Phenotypic prediction

The small sample size of our diallel and the general low frequency of deleterious SNPs precludes association-based approaches to evaluate the impact of variants on phenotypic variation. To alleviate this limitation, we conceived a haplotype-based genomic selection approach in which we use estimates of evolutionary constraint across the genome (Rodgers-Melnick *et al.* 2015) to sum the individual effects of deleterious alleles within IBD blocks under both an additive and dominant model (see Methods and Figure S5).

A Bayesian-based statistical method (BayesC) (Habier *et al.* 2011) using a 5-fold cross-validation approachwas was employed for model training. In general, average prediction accuracies were higher using the additive model (mean r = 0.81 and 0.49 for traits *per se* and BPH) than the dominant model (mean r = 0.70 and 0.42), and accuracies for heterosis traits were lower than for traits *per se* (Table S3). *is there a table we can cite that has all these?* **see edits** Incorporating evolutionary constraint information improved prediction accuracy for ASI and PHT *per se* under an additive model and for ASI under a dominant model (FDR < 0.05, Figure 3 A and B). GERP scores also improved prediction accuracies of heterosis (BPH) for GY under the additive model and DTP, DTS and TW under the dominant model (FDR < 0.05, Figure 3 C and D). *ref/link to supp table 3 please. also, do we need to include FDR here? what are the multiple tests the FDR is correcting for here?* **move table S3 to above. added FDR. FDR is for correcting multiple traits and multiple transformations.** To rule out the possible confounding of high GERP scores and genic annotations, we re-permuted the data using only deleterious (GERP > 0) genic SNPs. Though this resulted in fewer SNPs (316, 983), the model prediction accuracies remained significantly improved for GY *per se* under the additive model and for BPH of GY and PHT under the additive model (Figure S6 and Table S4).

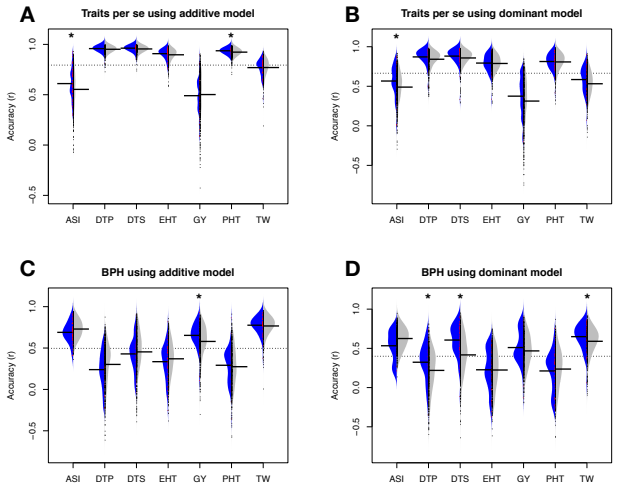

Figure 3 Beanplots of cross-validation accuracies using SNPs with positive GERP scores. Cross-validation experiments were conducted using selected SNPs and permuted data for traits *per se* (**A, B**) and BPH (**C, D**) under additive (**A and C**) and dominant (**B and D**) models. Accuraries from the real data were plotted on the left (blue) and permutation results on the right (grey). Horizotal bars indicate mean accuracies for each trait and the grey dashed lines indicate the overall mean accuracy. Stars indicate significantly (FDR < 0.05) higher cross-validation accuracies.

Posterior phenotypic variance explained and model comparisons

To learn why the prediction performace varied among traits *per se* and heterosis, we obtained the posterior variance explained by our models using the complete set of data. As shown in Figure 4, additive models explained more phenotypic variance for traits *per se* of DTP, DTS, EHT and PHT; but explained less phenotypic variance for heterosis (BPH) of ASI, GY and TW. In contrast, a larger proportion of the phenotypic variance could be explained by the dominant models for heterosis (BPH) of ASI, GY and TW. This difference was particularly striking for grain yield under the dominant model, where only 3% of the variance in trait *per se* could be explained but 61% of the variance in BPH was explained.

Heterosis transformations were largely determined by the accuracies of the parental phenotypes. To take the uncertainty of the parental phenotypes into consideration, we estimated combining ability from the hybrid population itself to investigate which modes of inheritance perform better than the null models. We extracted the breeding values estimated under both additive and dominant models using our haplotype blocks and incorporating GERP scores. We then applied the following models:

$$Y_{ij} = \mu + GCA_i + GCA_j + \varepsilon \quad (1)$$

$$Y_{ij} = \mu + GCA_i + GCA_j + G_{ij} + \varepsilon \quad (2)$$

$$Y_{ij} = \mu + GCA_i + GCA_j + SCA_{ij} + \varepsilon \quad (3)$$

$$Y_{ij} = \mu + GCA_i + GCA_j + SCA_{ij} + G_{ij} + \varepsilon \quad (4)$$

where Y_{ij} is the BLUE value of the hybrid crossed between the i^{th} inbred and j^{th} inbred; μ, the overall mean; GCA_i, the general combining ability of the i^{th} inbred; GCA_j, the general combining ability of the j^{th} inbred; SCA_{ij}, the specific combining ability of between the i^{th} and j^{th} inbreds; G_{ij}, breeding values estimated by our GS model for hybrid crossed between the i^{th} inbred and j^{th} inbred; ε, the model residuals.

Consistent with the previous analysis, haplotype blocks coded with the dominant mode of inheritance significantly improved model fitting for ASI and GY (equation 1 vs. equation 2, ANOVA P value < 0.05, Table S5). Comparison of model 3 and model 4 indicated that model 4 performed almost as well as 3 (ANOVA P value = **X**), indicating that specific combining ability captured most of the parental interactions and the current method could not detect higher order of interactions. *does model 2 outperform model 1? does 3 outperform 2? 4 should outperform 3 because it has extra parameters, but the text says "almost as well"?* **We are comparing [1 and 2], [3 and 4] only by adding breeding value estimated from our GERP GS model. I do not think this result add anything to our story. maybe we will ditch it?**

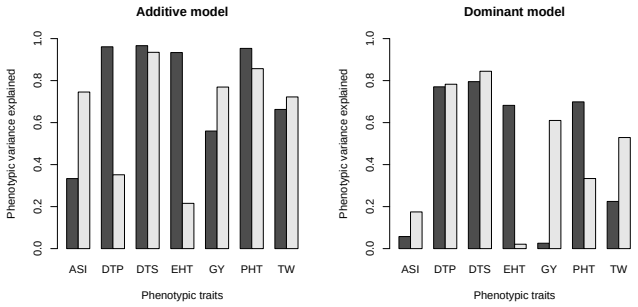

Figure 4 Posterior phenotypic variance explained by deleterious genic SNPs in IBD blocks using additive and dominant models. Dark color indicates trait *per se* and grey color indicates BPH.

Discussion

- do we support deleterious model of Mezmouk et al.? **yes**
- how do results match with heritability and heterosis? **and mode of inheritance**
- Indication for breeding? genomic selection using GERP and other annotation information.

In this study we have identified more than 500,000 deleterious SNPs in panel of elite maize lines. On average, each elite inbred line carries about 100,000 deleterious SNPs (GERP > 0). *cool, this should be mentioned here but also in the results. is there any big variation? some lines more than others? is mo17 worse than the pvps? given the comment below i added about linkage, can we calculate mean deleterious variants per cM? that would be sweet to know and to point out that there are tons around the centromere* Across lines, however, the majority of these deleterious mutations were maintained at low frequency, consistent with previous observations (Rodgers-Melnick *et al.* 2015). The large number of linked deleterious alleles present would likely make it hard to completely purge all such alleles through breeding efforts. Instead, breeders have devised a strategy — hybrid breeding — that may circumvent much of this problem via complementation. Indeed, results in this study show that prediction accuracies were higher for yield heterosis when information about deleterious SNPs was incorporated into the prediction model. Because there are likely

thousands of deleterious alleles involved in complementation, many with relatively small effects, traditional GWAS approaches with genome-wide thresholds of multiple testing are likely unable to detect such effects. Using a liberal significance threshold, however, our previous efforts nonetheless showed an enrichment for deleterious genic SNPs among associated markers for a number of traits (Mezmouk and Ross-Ibarra 2014).

Our models did not increase the prediction accuracies equally well for traits *per se* and their heterosis transformations. This is not surprising, however, given that the variation in genetic architectures of different phenotypic traits — flowering time, for example, appears primarily determined by many loci of small additive effect (Buckler *et al.* 2009), while **X** *better to use another maize example if we can rather than rice...* . Consistent with this, we observed that the additive model increased prediction accuracies for ASI as a trait *per se* and *ideally above use a phenotype we can compare in this way... dominant model increased the prediction accuacies for trait* per se *of GY.* One limitation of our current models is that we assume phenotypic traits are determined by complete additive or complete dominant effects. Traits with a mixture of additive and dominant casual loci may thus fail to be predicted. Another limitaiton is likely the population size used; we may simply not have enough power to predict traits with low heritability.

To test the simple complementation hypothesis, we developed a GS pipeline, which incorporated evolutionary conservation information in the GS model. As the genotyping cost keeps declining, GS gains its popularity in plant breeding (Desta and Ortiz 2014). Previous studies mainly focused on developing statistical approaches to improve the efficiency of GS model, however, none of the existing studies have taken prior biological information into consideration. It is known that SNP variations would have varied impacts depending on their genomic position (e.g.) **(CITE)** , biological function (e.g. transcription binding sites) and evolutionary conservation. The incorporation of GERP information under the Bayesian framework developed in this study is the first step towarding more meaningful models for further GS.

Acknowledgements

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Supporting Information

Table S1 BLUE values of the seven phenotypic traits. (https://github.com/RILAB/pvpDiallel/blob/master/manuscript/Figure_Table/Table_S1.trait_matrix.csv)

Table S2 General combining ability and specific combining ability of the seven phenotypic traits. (https://github.com/RILAB/pvpDiallel/blob/master/manuscript/Figure_Table/Table_S2.CA.csv)

Table S3 Cross-validation results using all genome-wide deleterious SNPs. (https://github.com/RILAB/pvpDiallel/blob/master/manuscript/Figure_Table/Table_S3_allsnps_FDR.csv)

Table S4 Cross-validation results using deleterious genic SNPs. (https://github.com/RILAB/pvpDiallel/blob/master/manuscript/Figure_Table/Table_S4_genicsnps_FDR.csv)

Table S5 ANOVA P values of model comparisons. (https://github.com/RILAB/pvpDiallel/blob/master/manuscript/Figure_Table/Table_S5_model_comp.csv)

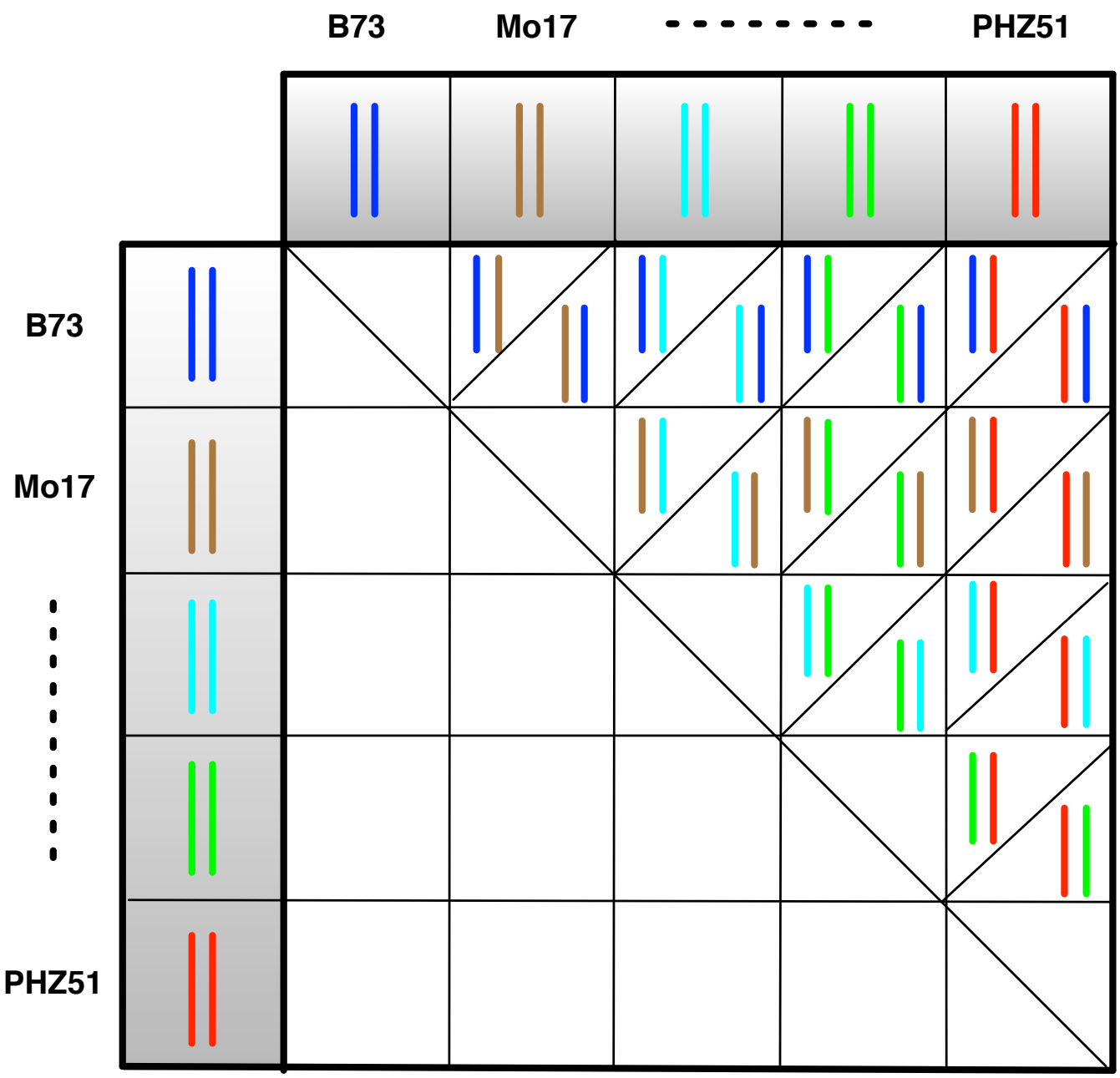
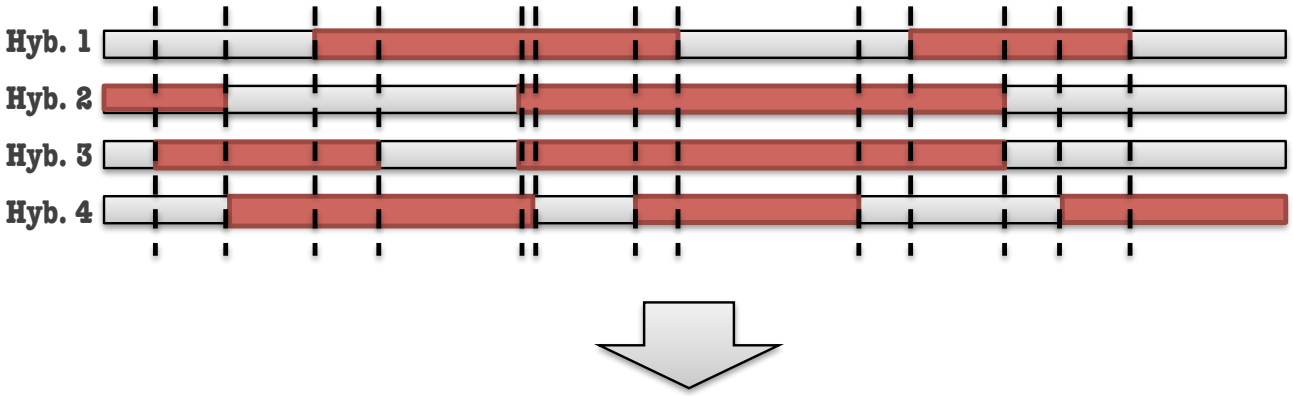


Figure S1 Twelve maize inbred lines were selected and crossed in a partial diallel fashion. Each inbred lines was used as both male and female and the resulting F1s were bulked. *do we need to modify this diagram now that we now reciprocal crosses were pooled?* **any other idea to re-design the figure? or ditch it?** *this isn't bad, but what deleting the bottom triangle completely rather than leave the cells blank?*



	HB1	HB2	HB3	HB4	HB5	HB6	HB7	HB8	HB9	HB10	HB11	HB12	HB13	HB14
Hyb1	0	0	0	1	1	1	1	1	0	0	1	1	1	0
Hyb2	1	1	0	0	0	1	1	1	1	1	1	0	0	0
Hyb3	0	1	1	1	0	1	1	1	1	1	1	0	0	0
Hyb4	0	0	1	1	1	1	0	1	1	0	0	0	1	1

Figure S2 Haplotype block identification using an IBD approach. In the upper panel, regions in red are IBD blocks identified by pairwise comparison of the two parental lines of a hybrid. The vertical dashed lines define haplotype blocks. In the lower panel, hybrid genotypes at each block are coded as heterozygotes (0) or homozygotes (1).

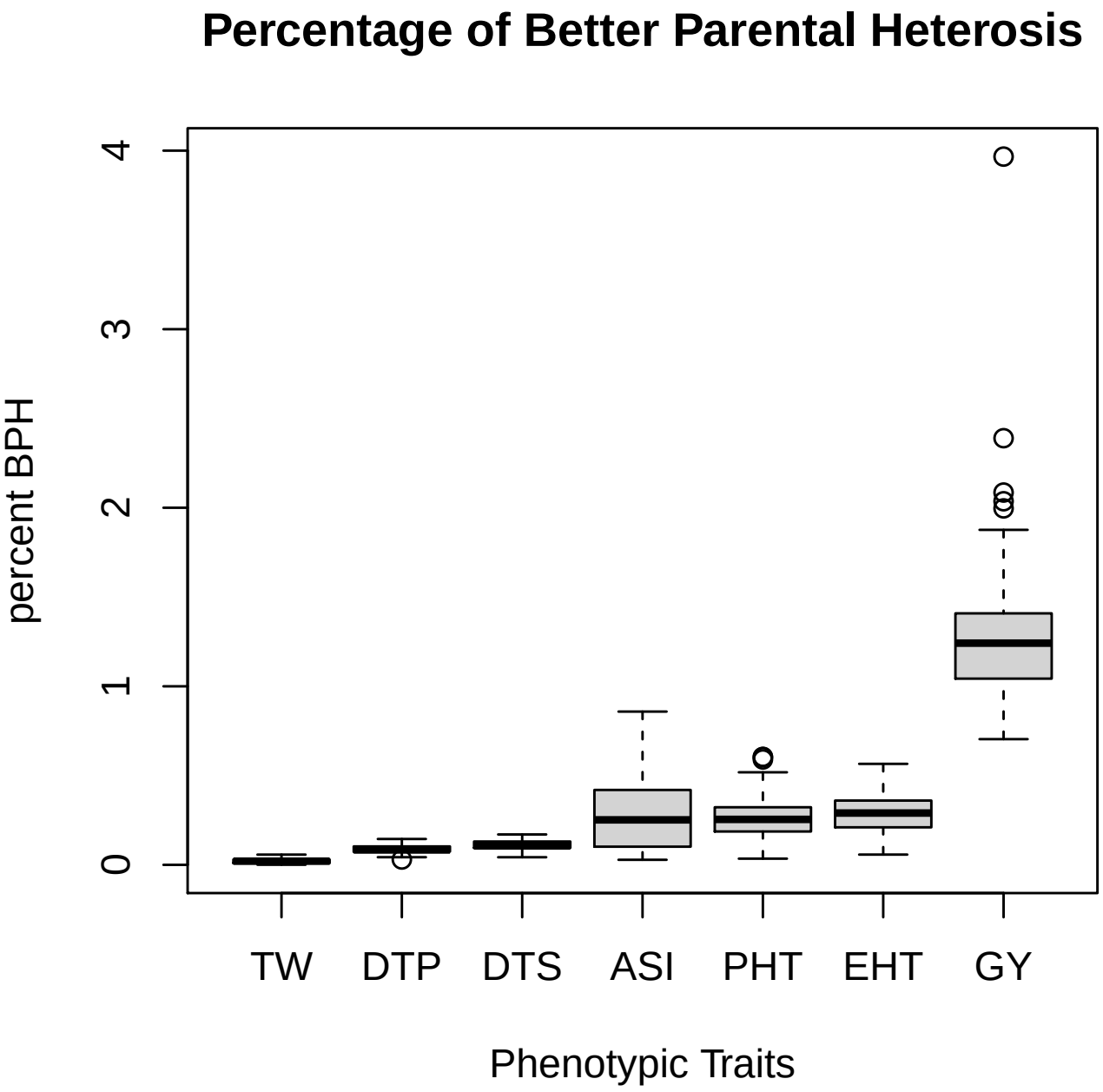


Figure S3 Boxplot of percent best parent heterosis (pBPH). In the plot, ASI was calculated using pBPHmin and the other six traits were calculated using pBPHmax.

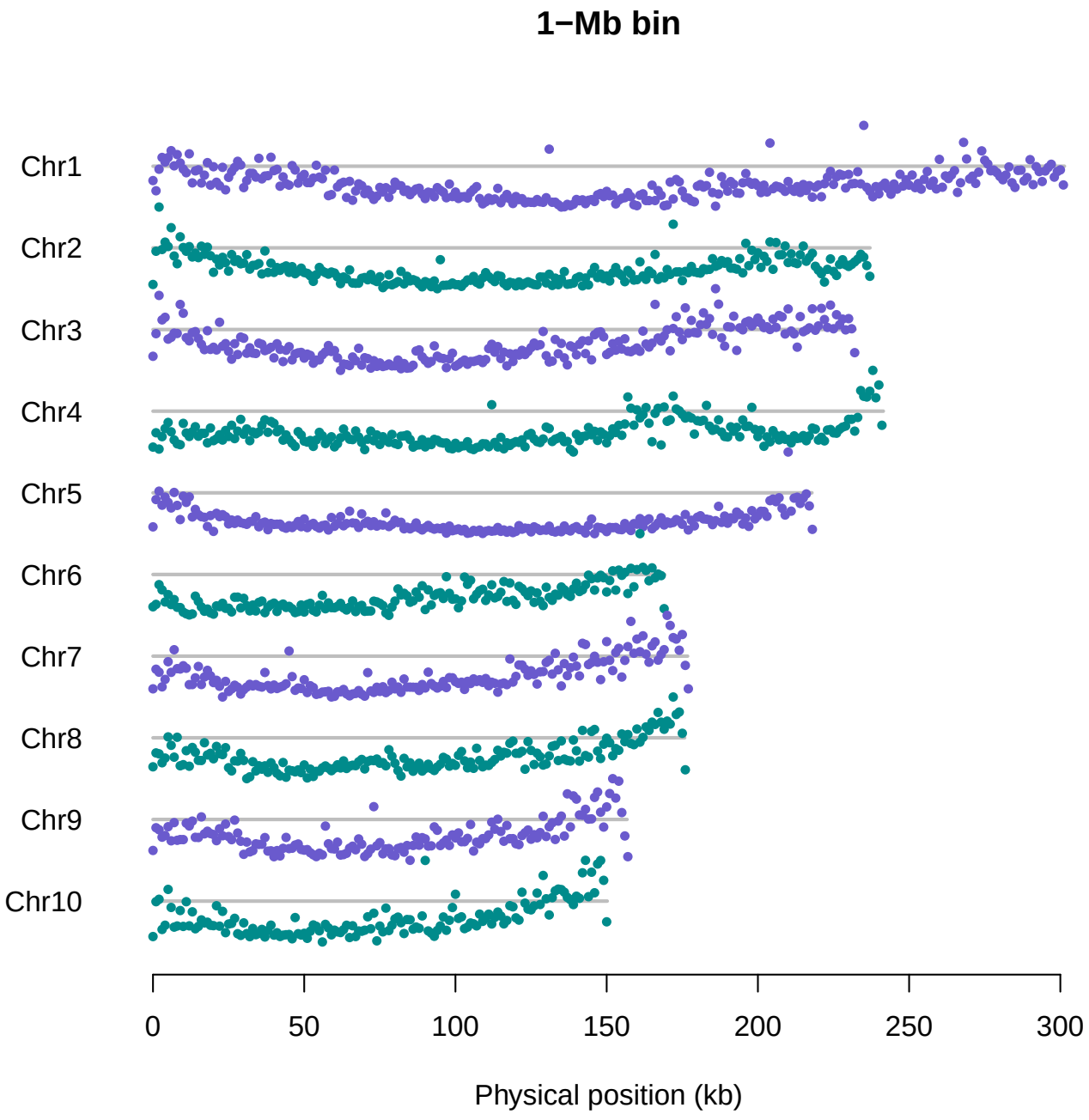


Figure S4 GERP score distribution across the genome. Shown are mean GERP scores in a 1-Mb bin region.

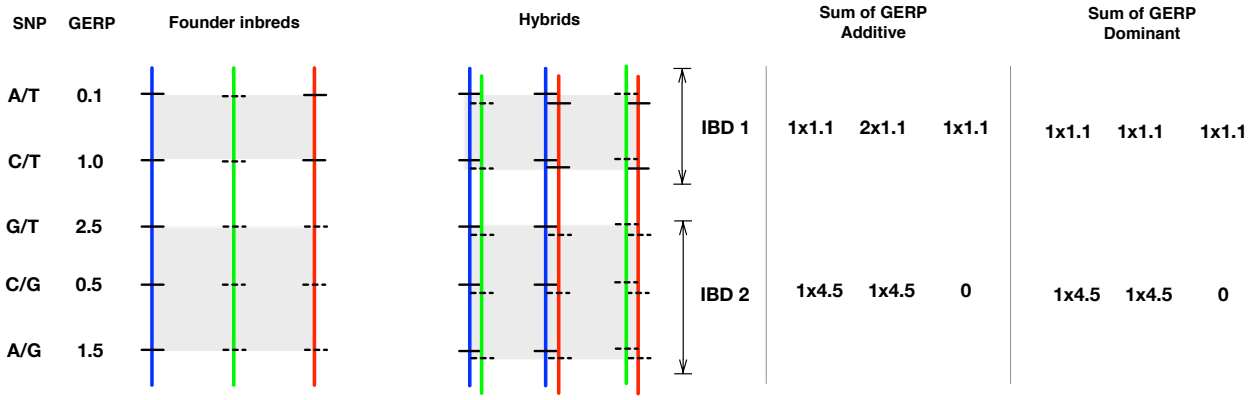


Figure S5 Incoporation of conservation information into IBD blocks. Regions of the genome that are identical by descent (IBD) among the 12 inbreds were identified using Beagle (Browning and Browning 2009). The GERP scores of SNPs in an IBD block were summed under both additive and dominant models. For a particular SNP with GERP score g, the homozygous non-reference genotype was assigned a value of $2g$, the heterozygote assigned a value of g, and the reference homozygote a value of 0. Under the dominant model, both the heterozygote and the non-reference homozygote were assigned a value of g, with the reference homozygote again assigned a value of 0.

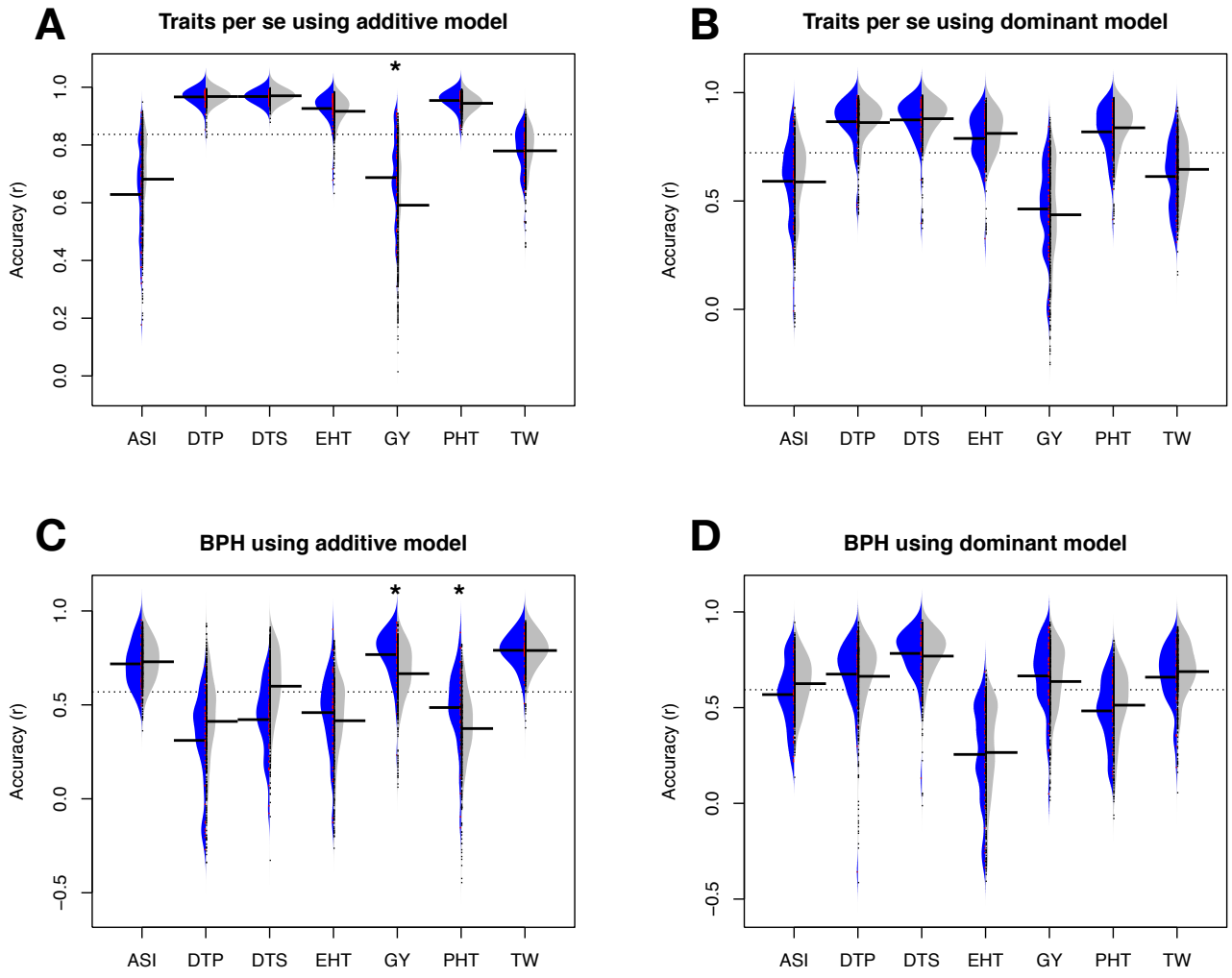


Figure S6 Cross-validation accuracies using genic SNPs. Cross-validation experiments were conducted using genic SNPs and compared to circular-shuffled data for traits *per se* (**A, B**) and pBPH (**C, D**) under additive (**A, C**) and dominant (**B, D**) models. Distirbutions show accuracty of prediction from real data (blue) and permutations (grey), with horizontal bars to indicate mean accuracy. Stars indicate significantly higher cross-validation accuracy for the real data. The average accuracy across all traits is shown with the grey dotted line.