

# Omics-based Hybrid Prediction in Maize

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## 1 **Key message**

2 Complementing genomic data with other "omics" predictors can increase the proba-  
3 bility of success for predicting the best hybrid combinations using complex agronomic  
4 traits.

## 5 **Abstract**

6 Accurate prediction of traits with complex genetic architecture is crucial for select-  
7 ing superior candidates in animal and plant breeding and for guiding decisions in  
8 personalized medicine. Whole-genome prediction (WGP) has revolutionized these  
9 areas but has inherent limitations in incorporating intricate epistatic interactions.  
10 Downstream "omics" data are expected to integrate interactions within and between  
11 different biological strata and provide the opportunity to improve trait prediction.  
12 Yet, predicting traits from parents to progeny has not been addressed by a combi-  
13 nation of "omics" data. Here, we evaluate several "omics" predictors — genomic,  
14 transcriptomic and metabolic data — measured on parent lines at early developmen-  
15 tal stages, and demonstrate that the integration of transcriptomic with genomic data  
16 leads to higher success rates in the correct prediction of untested hybrid combinations  
17 in maize. Despite the high predictive ability of genomic data, transcriptomic data  
18 alone outperformed them and other predictors for the most complex heterotic trait,  
19 dry matter yield. An eQTL analysis revealed that transcriptomic data integrate  
20 genomic information from both, adjacent and distant sites relative to the expressed  
21 genes. Together, these findings suggest that downstream predictors capture phys-

22 iological epistasis that is transmitted from parents to their hybrid offspring. We  
23 conclude that the use of downstream "omics" data in prediction can exploit impor-  
24 tant information beyond structural genomics for leveraging the efficiency of hybrid  
25 breeding.

## 26 **Conflict of Interest**

27 The authors declare that they have no conflict of interest.

## 28 Introduction

29 Hybrid breeding, which entails crossing of lines from two genetically distant germplasm  
30 collections — called heterotic groups (Melchinger and Gumber, 1998) — has emerged  
31 as a prime strategy to meet demands for a sustainable intensification of agricultural  
32 production (Duvick, 2005). However, unlocking the full potential of hybrid breeding  
33 requires accurate prediction methods to efficiently identify the superior candidates  
34 out of the millions of possible hybrids that could potentially be produced in each cy-  
35 cle of an ordinary-sized breeding program. With the advent of the doubled haploid  
36 (DH) technology (Wedzony et al., 2009) this prediction problem has become even  
37 more challenging because, based on breeder's experience, the vast majority ( $\approx 90\%$ )  
38 of competing lines in each heterotic group are "new" lines without any phenotypic  
39 records on hybrid progeny from previous breeding cycles. Consequently, among all  
40 hybrid combinations possible between lines from two heterotic groups, about 81%  
41 are T0 hybrids, 18% are T1 hybrids and 1% are T2 hybrids having zero, one or  
42 two parents, respectively, that have been previously tested in other hybrid combina-  
43 tions. Preselection of a few hundred of the most favorable hybrids with high success  
44 rate could significantly reduce the labor-intensive and time-consuming field-testing  
45 (Kadam et al., 2016, Xu et al., 2016). This could greatly impact the efficiency of  
46 hybrid breeding and boost the annual selection gain (Longin, Mi and Würschum,  
47 2015).

48 Whereas yield and other heterotic traits of hybrids are generally poorly predicted  
49 by the performance of their parent lines (Melchinger and Gumber, 1998), WGP has  
50 emerged as a major tool for tackling this challenge (Massman et al., 2013, Technow

51 et al., 2014). Nevertheless, there is evidence that, even with complete sequence in-  
52 formation, genomic prediction may not capture complex interactions between genes  
53 and downstream regulation, which act through the entire cascade from genotype  
54 to phenotype (Dalchau et al., 2011, Zhu et al., 2012, Rudd et al., 2015, Ritchie,  
55 Holzinger, Li, Pendergrass and Kim, 2015). Most studies have evaluated predic-  
56 tive ability by looking at only one kind of endophenotype (intermediaries between  
57 genotype and phenotype (Gottesman and Gould, 2003, Mackay, Stone and Ayroles,  
58 2009) such as the transcriptome (Swanson-Wagner et al., 2006, Zenke-Philippi et al.,  
59 2016, Xu et al., 2016) or the metabolome (Riedelsheimer et al., 2012, Xu et al., 2016,  
60 Dan et al., 2016). The integration of different endophenotypic and genomic data is  
61 expected to reflect more closely the variability across genotypes than genomic data  
62 alone (Mackay, Stone and Ayroles, 2009, Patti, Yanes and Siuzdak, 2012, Civelek  
63 and Lusi, 2014). Two recent studies that integrated multiple biological strata in  
64 predicting breast cancer risk (Vazquez et al., 2016) and performance of maize in-  
65 bred lines (Guo et al., 2016), respectively, demonstrated the benefit of this strategy.  
66 However, unlike forecasting clinical or agronomic traits from endophenotypes of the  
67 same genotype, hybrid breeding requires the prediction of the genotypic values (GV)  
68 of hybrid progeny based on parental information. To achieve this objective, we used  
69 the BLUP approach — originally developed in animal breeding (Henderson, 1984) —  
70 for the more complex setting of hybrids between parents from two heterotic groups  
71 (Bernardo, 1996, Massman et al., 2013). Here, we measured endophenotypes of par-  
72 ent lines to forecast the GV of T0, T1 and T2 hybrid progeny by using prediction  
73 equations trained with "omics" information from other parent lines and phenotypic

74 information on their hybrid offspring.

## 75 **Materials and Methods**

### 76 **Genetic material and phenotyping**

77 The entire genetic material consisted of a set of 1,536 hybrids, denoted as  $H_{Tot}$ ,  
78 produced in 16 factorial mating designs between 142 Dent and 103 Flint lines from  
79 the maize breeding program at the University of Hohenheim, on which agronomic  
80 data for silage maize production, as well as pedigree and genomic data were available.  
81 A subset of this material, albeit from different trials, has been used for genomic  
82 prediction of traits related to grain maize production (Technow et al., 2014). For  
83 hybrid prediction, we used a core set  $H \subset H_{Tot}$  of 617 hybrids, produced in six  
84 factorials with hybrid sets  $H_{FAC(i)} (i = 1, 2, \dots, 6; H = \bigcup_{i=1}^6 H_{FAC(i)})$  from crosses  
85 between 57 Dent and 41 Flint inbred lines, denoted as  $D = \{1, 2, \dots, 57\}$  and  $F =$   
86  $\{1, 2, \dots, 41\}$  (File S1). All hybrids were evaluated in field experiments at three  
87 or more agro-ecologically diverse locations across Germany. In the trials of each  
88 factorial, which included at least five common check genotypes, the entries were  
89 randomized in  $\alpha$  lattice designs and planted in 2-row plots. Dry matter yield (DMY,  
90  $t/ha$ ) and dry matter content (DMC, %) of whole-plant aboveground biomass were  
91 determined by established procedures (Riedelsheimer et al., 2012). For quality traits,  
92 contents of fiber (ADF, %), fat (FAT, %<sub>0</sub>), protein (PRO, %<sub>0</sub>), starch (STA, %), and  
93 sugars (SUG, %<sub>0</sub>) in dry matter were measured in the harvested plant material using  
94 calibrated near-infrared spectroscopy (NIRS; Grieder et al. (2011), File S1).

## 95 **Pedigree-based relationship coefficients**

96 Coancestry coefficients were calculated using SAS (version 9.4, SAS Institute) for all  
97 possible pairs of lines in each heterotic group according to established rules (Falconer  
98 and Mackay, 1996) under the following assumptions (Cox, Murphy and Rodgers,  
99 1986): (i) all lines in a pedigree are genetically homogeneous and homozygous, (ii)  
100 pairs of genotypes with no known common parentage are unrelated, and (iii) a line  
101 derived from a cross or backcross obtained a proportional fraction of the genome from  
102 each parent, as expected under Mendelian inheritance in the absence of selection.

## 103 **Genotyping**

104 Genotyping of all inbred lines was performed with the Illumina SNP chip MaizeSNP50  
105 (Ganal et al., 2011). After performing a commonly used quality check (Technow  
106 et al., 2014) and imputation of missing data (Browning and Browning, 2009), a total  
107 of 21,565 polymorphic SNPs was available and used for all further analyses.

## 108 **Metabolite profiling**

109 Seedlings of all parental inbred lines were grown under controlled conditions inside  
110 climate chambers to quantify the metabolite profiles of their roots 3.5 days after  
111 sowing, as detailed by de Abreu e Lima et al. (2017). The experiment was laid out  
112 as a randomized incomplete block design with replicated germination boxes. For leaf  
113 metabolic profiles (known metabolites and unannotated chromatographic peaks), a  
114 field experiment was carried out in an  $\alpha$  lattice design with two replications at one



115 location in southern Germany in the spring of 2012. Excision of leaves at the third  
116 leaf stage was performed according to an established protocol (Riedelsheimer et al.,  
117 2012) 28 days after sowing, in the afternoon of a cloudy day, and finalized within  
118 45 minutes for the entire experiment. For both profiling procedures, all material  
119 was transferred directly into containers with dry ice and then into liquid nitrogen to  
120 quench metabolic activity.

## 121 **Transcriptome profiling**

122 For transcriptome profiling, five seeds per parent line were taken from the same  
123 seed lot as used for metabolite profiling and laid out inside a climate chamber in  
124 a randomized complete block design with five replications. Seedlings were sampled  
125 seven days after sowing, snap-frozen in liquid nitrogen, and stored at -80°C until use.  
126 Prior to mRNA extraction, roots from all replicates of a genotype were pooled and  
127 homogenized. A custom 2K-microarray (GPL22267) was assembled from a subset  
128 of the 47K maize oligonucleotide array (GPL6438). Two-color hybridizations were  
129 carried out separately for each of the six factorials using interwoven loop designs (Kerr  
130 and Churchill, 2001). The average number of shared genotypes between factorials  
131 was 4.5 and ranged from 2 to 10.

## 132 **Statistical analysis of agronomic traits**

133 Agronomic data were analyzed in two stages, following Technow et al. (2014) by  
134 accounting for year, location, field replication, block and genotype effects as well as  
135 their interactions (File S1). In the first stage, and separately for each environment,

136 best linear unbiased estimates (BLUEs) of the  $\alpha$  designs were computed for every  
137 hybrid using REML-based linear mixed-model analyses. In the second stage, BLUEs  
138 were computed for all hybrids in  $H_{Tot}$ . The BLUEs of hybrids in the core set  $H$  served  
139 as response variables in our hybrid prediction models and cross-validation routines.  
140 For all predictions we used computationally efficient best linear unbiased predictor  
141 (BLUP) models, which have the same properties as those of a selection index because  
142 we previously accounted for fixed effects (Mrode (2014), pp. 34, 311, 312). For  
143 general and specific combining abilities (GCA and SCA) of parent lines, we used  
144 ASReml (Butler et al., 2009) to compute best linear unbiased predictors (BLUPs),  
145 variance components ( $\sigma_{GCA^D}^2$ ,  $\sigma_{GCA^F}^2$ ,  $\sigma_{SCA}^2$ ) and entry-mean heritabilities ( $H^2$ ) of all  
146 hybrids in  $H_{Tot}$ , treating all effects in the model as random. The covariance matrices  
147 of the GCA and SCA effects were defined by multiplying the variance components  
148 with their respective genomic relationship matrices (File S1).

## 149 **Statistical analysis of endophenotypes**

150 Raw data were normalized using established procedures for metabolites (van den Berg  
151 et al., 2006) and transcripts (Smyth and Speed, 2003, Ritchie et al., 2007). From these  
152 data, we obtained BLUEs for metabolite levels and transcript abundance of each line  
153 using REML-based mixed-model analyses. The statistical models for the analysis of  
154 metabolite profiles accounted for various experimental effects as detailed by de Abreu  
155 e Lima et al. (2017). After applying quality checks and computing BLUEs, 92 leaf  
156 metabolic analytes and 283 root metabolic analytes remained for further analyses.  
157 BLUEs for transcriptomic data were computed using the R-package *limma* (Ritchie,

158 Phipson, Wu, Hu, Law, Shi and Smyth, 2015) in reference to established protocols  
159 (Smyth and Speed, 2003, Ritchie et al., 2007, Frisch et al., 2010). The design matrix  
160 for the linear model was based on the dye-labeling of a reference genotype. To account  
161 for possible differences between the microarrays, replicates of some genotypes across  
162 at least two factorials were included, and modeled through a fixed effect term. All  
163 gene expression values were subsequently computed, based on the log-ratio relative  
164 to this common genotype (Smyth, 2004). In total, 1,323 gene expression profiles were  
165 available. Repeatabilities ( $w^2$ ) were estimated for each endophenotype at the inbred  
166 line level using the same models as for the computation of BLUEs, but treating the  
167 genotype effect as random. This analysis was performed jointly for the Dent and  
168 Flint lines allowing for different means and heterogeneous genotypic variances of the  
169 heterotic groups, but assuming a common error variance. Variance components were  
170 estimated by Gibbs sampling using the *R* package *MCMCglmm* (Hadfield, 2010).

## 171 Prediction models and model evaluation

172 Predictions of hybrid performance were compared on the basis of the core set of  
173 hybrids  $H$  and the corresponding sets of parent lines  $D$  and  $F$  on which data for  
174 all five predictors (P, pedigree; G, genomic; T, transcriptomic; L, leaf metabolic;  
175 R, root metabolic data) were available with the exception of data on a few lines  
176 missing at random for R due to fungal contamination. The matrices  $\mathbf{W}_D$  and  $\mathbf{W}_F$   
177 are matrices of standardized feature measurements for the various predictors (G, T,  
178 L, R). The matrix  $\mathbf{W}$  has dimension 'number of parent lines in the corresponding  
179 heterotic group' ( $n_D = 142$ ,  $n_F = 103$ ) times 'number of features' ( $w_G = 21,565$ ,

180  $w_T = 1,323$ ,  $w_L = 92$ ,  $w_R = 283$ ). The columns in  $\mathbf{W}_D$  and  $\mathbf{W}_F$  are centered and  
 181 standardized to unit variance, respectively.

The kernels pertaining to each predictor and lines from each heterotic group — corresponding to genomic relationship matrices in the case of SNPs — can then be defined as

$$\mathbf{G}_D = \frac{1}{W} \mathbf{W}_D \mathbf{W}_D^\top, \quad \mathbf{G}_F = \frac{1}{W} \mathbf{W}_F \mathbf{W}_F^\top, \quad (1)$$

182 where  $W$  denotes the number of features (VanRaden, 2008). In the case of pedi-  
 183 gree data (P), coancestry coefficients were used directly for  $\mathbf{G}_D$  and  $\mathbf{G}_F$ , respectively.

184 The universal model for GCA and SCA effects was:

$$\mathbf{y} = \mu + \sum_{c=1}^C \mathbf{Z}_D \mathbf{g}_{Dc} + \sum_{c=1}^C \mathbf{Z}_F \mathbf{g}_{Fc} + \sum_{c=1}^C \mathbf{Z}_S \mathbf{s}_c + \epsilon, \quad (2)$$

185 where  $\mathbf{y}$  is the vector of observed hybrid performance (BLUEs),  $\mu$  is the fixed  
 186 model intercept,  $\mathbf{Z}_D$  is the corresponding design matrix associating the random GCA  
 187 effects of the lines in  $D$  ( $\mathbf{g}_{Dc}$ ) with  $\mathbf{y}$ ,  $\mathbf{Z}_F$  is the corresponding design matrix asso-  
 188 ciating the random GCA effects of the lines in  $F$  ( $\mathbf{g}_{Fc}$ ) with  $\mathbf{y}$  and  $\mathbf{Z}_S$  is a design  
 189 matrix associating the SCA effects ( $\mathbf{s}_c$ ), pertaining to hybrid combinations for the  
 190  $c$ -th predictor data type with the corresponding hybrid measurements in  $\mathbf{y}$ . Thus,  
 191 the model in Eq. 2 can accommodate just one ( $C = 1$ ) or multiple ( $C > 1$ ) predictors  
 192 simultaneously. The random effects ( $\mathbf{g}_{Dc}$  and  $\mathbf{g}_{Fc}$ ) have expectation zero and covari-  
 193 ance matrices equal to  $\mathbf{G}_{Dc} \sigma_{GCA^{Dc}}^2$  and  $\mathbf{G}_{Fc} \sigma_{GCA^{Fc}}^2$  for the GCA effects of the Dent  
 194 and Flint lines, respectively,  $\mathbf{S}_c \sigma_{SCA^c}^2$  for the SCA effects and  $\mathbf{I} \sigma_\epsilon^2$  for the residual er-  
 195 ror. For each combination between crosses of lines  $i \times k$  and  $j \times l$ , the corresponding

196 elements in  $\mathbf{S}_c$  were obtained as the product of the respective elements  $f_{ij}$  in  $\mathbf{G}_{Dc}$  and  
197  $f_{kl}$  in  $\mathbf{G}_{Fc}$ , respectively (Schnell, 1965, Henderson, 1985, Bernardo, 1996, Massman  
198 et al., 2013, Technow et al., 2014, Jiang and Reif, 2015) (File S1). Note that, in  
199 the majority of cases, only GCA effects were considered. In the absence of epistasis,  
200 this model is equivalent to a feature model accounting for dually defined additive  
201 effects in each heterotic group and dominance effects between them. Extensions of  
202 the single-predictor models were made by adding GCA and SCA effects for any ad-  
203 ditional predictor assuming stochastic independence of effects. In order to obtain  
204 unbiased estimates of the predictive ability and to compare different models and pre-  
205 dictor combinations, following Technow et al. (2014), we devised a cross-validation  
206 (CV) scheme, stratified by the parent lines and using 1,000 runs (CV1000, File S1).  
207 All prediction models were implemented using the *R* package *BGLR* (Pérez and de  
208 Los Campos, 2014).

## 209 **Comparison of predictive abilities**

210 Predictive abilities were obtained by calculating Pearson correlations between pre-  
211 dicted ( $\hat{y}$ ) and observed phenotypes ( $y$ ), separately for three test set partitions (T0,  
212 T1 and T2 hybrids). For each CV run, the training and validation sets were stored to  
213 ensure the validity of comparisons between any predictor and combinations thereof.  
214 For any two predictors, say  $A$  and  $B$ , we then have orthogonal vectors with predictive  
215 abilities  $r_A$  and  $r_B$  of length 'number of cross validation runs'.

## 216 **Evaluation of a pre-selection bias in transcriptomic data**

217 A custom 2K-microarray (GPL22267) was assembled from a subset of the 47K maize  
218 oligonucleotide array (GPL6438), based on association of genes with hybrid perfor-  
219 mance or mid-parent heterosis for grain yield and grain dry matter content of maize.  
220 These two traits were evaluated in separate grain-yield trials with hybrids from fac-  
221 torial  $H_{\text{FAC}(1)}$  (Frisch et al. (2010), Thiemann et al. (2010), File S1). To ensure that  
222 no pre-selection bias was introduced in hybrid prediction using these transcriptomic  
223 data, we compared predictive abilities among the various predictors when excluding  
224  $H_{\text{FAC}(1)}$  from the entire set  $H$ .

## 225 **Association mapping**

226 For each of the seven agronomic traits, we performed a genome-wide association study  
227 (GWAS) with GCA effects of all 142 Dent and 103 Flint parent lines as response  
228 variables using the EMMAX-method (Kang et al., 2010) as implemented in *cpgen*  
229 (Heuer, 2015). To avoid using the marker data twice, GCA effects were calculated  
230 using only pedigree information. Furthermore, an eQTL analysis was carried out  
231 to examine statistically significant associations between genomic and transcriptomic  
232 data for the parent lines ( $D$  and  $F$ ) of the core set  $H$  plus five additional lines. This  
233 was accomplished in the same way as in the GWAS for agronomic traits, but here the  
234 BLUPs of the transcriptomic data of each mRNA were used as the response variables.  
235 Associations in each GWAS were declared statistically significant at  $\alpha = 0.05$  after  
236 Bonferroni correction.

## 237 **Probability of success**

238 Following Robson, Powers and Urquhart (1967), we calculated the probability of suc-  
239 cess ( $P[r, \beta]$ ) that a hybrid, selected at random from the upper  $\beta$  percent fraction of  
240 the distribution of predicted values for predictor A, has a phenotypic value contained  
241 in the upper  $\beta$  percent of the distribution of observed values. Denoting the predictive  
242 ability of a given predictor by  $r$ , this conditional probability was calculated assuming  
243 a bivariate normal distribution

$$\begin{bmatrix} \hat{y} \\ y \end{bmatrix} \sim N \left( \begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 1 & r \\ r & 1 \end{bmatrix} \right). \quad (3)$$

244 The required integrals were solved within the *R* statistical environment using the  
245 *mvtnorm* package (Genz et al., 2017).

## 246 **Principal component analysis**

247 Principal component analyses (PCA) were carried out to examine whether differ-  
248 ent predictors can distinguish between Dent and Flint parent lines and to explore  
249 whether subpopulations exist within either hetrotic group. Prior to each PCA, all  
250 variables were scaled and centered. Clusters represent two component mixtures of  
251 bivariate t-distributions, which were estimated using Maximum Likelihood. Ellipses  
252 were drawn based on the 0.95 quantiles of the respective bivariate t-distributions.  
253 Unless stated otherwise, all statistical analyses were carried out inside the *R* envi-

254 ronment for statistical computing (R Core Team, 2016).

## 255 **Data availability**

256 The data and the code used to analyze the data are available upon request.



## 257 Results

### 258 Agronomic data

259 Mean values of the 1,536 hybrids for the seven evaluated agronomic traits, relevant  
260 for animal feed and biogas production, were of the same magnitude as reported  
261 by Riedelsheimer et al. (2012) and Grieder et al. (2011). For all traits,  $\sigma_{GCA^D}^2$  and  
262  $\sigma_{GCA^F}^2$ , describing the main effects of the parents from each heterotic group, together  
263 explained more than 93% of the genotypic variance among hybrids (Table 1). Heri-  
264 tabilities were moderate to high for all agronomic traits, indicating a high precision  
265 of field experiments and data collection (Table 1).

266 [Table 1 about here.]

### 267 Predictor data

268 Repeatabilities ( $w^2$ ) for endophenotypes varied considerably in both groups of par-  
269 ents (Fig. S1a) with average values ranging from 0.31 to 0.41, except for transcrip-  
270 tomic data in Flint material where the average repeatability was only 0.18. Never-  
271 theless, in the latter case, 291 out of 1,323 transcripts still exceeded a threshold of  
272  $w^2 = 0.4$ .

273 Dent and Flint lines were clearly separated in principal component analyses of  
274 genomic and transcriptomic data (Fig. 1a) without signs of subpopulations within  
275 either group. However, they overlapped for leaf metabolic and, to an even greater  
276 extent, for root metabolic data. Off-diagonal elements of the kernels  $\mathbf{G}_D$  and  $\mathbf{G}_F$ ,  
277 respectively, showed moderate correlations between genomic and transcriptomic data

278 ( $\rho_D \approx 0.56, \rho_F \approx 0.44$ , Fig. S2). Correlations between the off-diagonal elements of  
279 the  $\mathbf{G}$ -matrices were highest for the comparison between genomic and pedigree data  
280 ( $\rho_D \approx 0.72, \rho_F \approx 0.63$ ). Intriguingly, the associations between the  $\mathbf{G}$ -matrices for  
281 the root and leaf metabolic data were very low ( $\rho_D \approx 0.12, \rho_F \approx 0.06$ ).

282 [Figure 1 about here.]

283 We observed high median pairwise linkage disequilibrium (LD) between SNP  
284 markers ( $r^2 \approx 0.39$  in Dent and  $r^2 \approx 0.37$  in Flint material) at a distance of  $\Delta\text{Mbp} \leq$   
285 0.125 (Fig. 1b). After an initial drop in  $r^2$  for  $\Delta > 0.125$ , substantial long-range  
286 LD remained. Large differences in allele frequencies in the two heterotic groups were  
287 present for 57% of SNPs (Fig. 2a,b) — particularly in the telomeric regions of the  
288 genome. An eQTL analysis performed with the parent lines suggests that transcript  
289 abundance integrates variegated genetic information given the fact that i) on the  
290 same chromosome, significant associations not only occurred between adjacent but  
291 also between distant pairs of expressed genes and SNPs and ii) 50% of the significant  
292 associations ( $\alpha = 0.05$ , Bonferroni-corrected) occurred between expressed genes and  
293 SNPs on different chromosomes (Fig. 2).

294 [Figure 2 about here.]

## 295 Predictive abilities

296 Assuming a polygenic architecture for all traits, as suggested by results from a GWAS  
297 (Fig. S3), we chose the best linear unbiased predictor (BLUP) method as a baseline  
298 for prediction of T0, T1 and T2 hybrids. Given that we corrected for fixed effects

299 in advance, this method corresponds to a selection index. A cross-validation scheme  
300 with 1,000 runs (CV1000), stratified by the parent lines, was devised (File S1, Fig.  
301 S4). Our main emphasis was on predicting T0 hybrids given the fact that they  
302 constitute the majority of possible hybrids in practical breeding programs (Kadam  
303 et al., 2016).

304 For predictive abilities ( $r$ ) of T0 hybrids, transcriptomic data alone were the best  
305 predictor for the most complex and highly heterotic trait, DMY, as well as for PRO  
306 (Fig. 3a). With transcriptomic data, the predictive ability  $r$  for DMY was 14.9%  
307 higher than for genomic data, resulting in an 85% increase in the probability of  
308 successfully selecting the best hybrid candidates  $P[r, \beta]$  for  $\beta = 0.01\%$  (Fig. 3b).  
309 This selection intensity corresponds to picking the top 100 out of  $10^6$  predicted  
310 hybrids for production and intensive testing in field trials.

311 [Figure 3 about here.]

312 Compared to other individual predictors,  $r$  obtained with genomic data alone  
313 were higher for FAT and SUG. Root metabolites displayed moderate to high predic-  
314 tive abilities for DMY and FAT, but did not perform well otherwise. Leaf metabo-  
315 lites performed relatively poorly for all traits. Regardless of the trait, combinations  
316 of genomic and transcriptomic information displayed robust and consistently high  
317 predictive abilities. Except for PRO, incorporating additional endophenotypes as  
318 predictors into our models did not yield notable improvements but remained at the  
319 same level compared to combining genomic and transcriptomic data. Incorporating  
320 SCA effects into our models did not further improve predictive abilities (Fig. S5).  
321 Results for the combination of other predictors with metabolic data are not presented

322 because no improvement of predictive abilities over the combination of genomic data  
323 with transcriptomic data and pedigree data could be achieved. Finally, we assessed  
324 the influence of the number of SNPs and mRNAs on predictive abilities. For genomic  
325 data, a subset of 5,000 SNPs already yielded the same predictive ability as when us-  
326 ing the entire available set. For transcriptomic data, the predictive ability improved  
327 only marginally with subsets larger than 50% of the available transcripts (Fig. S6).

## 328 Discussion

### 329 A paradigm shift in hybrid breeding

330 Hybrid breeding programs are generally based on genetically divergent heterotic  
331 groups. Their use enables a better exploitation of heterosis when conducting crosses  
332 between them (Melchinger and Gumber, 1998) and is expected to reduce the ratio of  
333 specific to general combining ability variance ( $\sigma_{SCA}^2 : \sigma_{GCA}^2$ ) in the crosses, thereby  
334 allowing for the selection of hybrids largely on the basis of GCA of their parent lines  
335 (Reif et al., 2007). However, obtaining accurate estimates of GCA requires the eval-  
336 uation of new lines in combinations with testers from the opposite heterotic group  
337 in multi-environment field trials. The promise of hybrid prediction is to accelerate  
338 breeding programs by skipping a large share of these tests in favor of selecting the  
339 most promising hybrids before they are even produced (Technow et al., 2014). This  
340 approach involves the prediction of an impressive number of putative hybrid candi-  
341 dates ( $n^2$ ) using predictor data collected on only  $2n$  parent lines. Crucial for hybrid  
342 prediction are predictors, which not only reflect the relationship between parental  
343 inbred lines but also the interaction of the two parental genomes in their hybrid  
344 progeny.

345 **Heterotic groups** Because of genetic drift and selection for hybrid performance,  
346 allele frequencies are expected to diverge in the two heterotic groups, thereby en-  
347 larging their genetic distance (Falconer and Mackay, 1996, Reif et al., 2007, Larièpe  
348 et al., 2017). Consistent with this hypothesis and two pilot studies with U.S. maize

349 lines (Gerke et al., 2015, Hall et al., 2016), Dent and Flint lines in our study were  
350 clearly separated in principal component analyses of genomic and transcriptomic  
351 data. With large differences in allele frequencies  $p^I$  and  $p^{II}$  in the two heterotic  
352 groups, as observed for 57% of SNPs, dominance variance  $\sigma_D^2$  becomes very small  
353 because it is a function of the product  $p^I(1-p^I)p^{II}(1-p^{II})$  (Stuber and Cockerham  
354 (1966), File S1).

355 Dominance variance ( $\sigma_D^2$ ) is the main component contributing to the variance  
356 of the specific combining ability effects ( $\sigma_{SCA}^2$ ), describing all types of interactions  
357 among the parental genomes in hybrid combinations. It was therefore not surpris-  
358 ing that the variances of the general combining ability (GCA) effects ( $\sigma_{GCA^D}^2$  and  
359  $\sigma_{GCA^F}^2$ ), describing the main effects of the parents from each heterotic group, to-  
360 gether explained more than 93% of the genotypic variance among hybrids for agro-  
361 nomic traits, which is consistent with earlier studies on silage maize of the Dent  $\times$   
362 Flint heterotic pattern (Geiger, Melchinger and Schmidt, 1986, Argillier, Méchin and  
363 Barrière, 2000). While the magnitude of SCA effects was trait specific, it was low for  
364 all observed traits, which is in agreement with previously reported values for yield  
365 and quality traits in silage maize (Grieder et al., 2012). The importance of GCA in  
366 our material was further corroborated by merely marginal differences in predictive  
367 abilities between models using only GCA effects and those that additionally incor-  
368 porated SCA effects (Fig. S5). Nevertheless, in crops such as wheat, with yet no  
369 clearly defined heterotic groups (Zhao et al., 2015) and greater importance of SCA,  
370 inclusion of SCA effects in the model should improve predictive abilities.

371 **Properties of well-established predictors** While pedigree data reflect the ex-  
372 pected relationship between genotypes, they do not necessarily depict their realized  
373 relationship. Genomic data and downstream endophenotypes offer to improve upon  
374 this pedigree-based approximation by more closely mirroring the transmission of  
375 genes between genotypes and their interactions. Genomic data have the advantage  
376 of reliably capturing Mendelian sampling, thereby improving pedigree-based predic-  
377 tion for many traits. However, genomic data alone may not be the final answer for  
378 the prediction of complex traits for two major reasons: First, the number of samples  
379 in most studies is considerably smaller than the number of genetic markers or even  
380 nucleotides of a genome. This implies that just modeling additive effects already  
381 necessitates shrinkage of effects. More importantly, however, interactions between  
382 loci throughout the genome can be frequent (Brem et al., 2005, Brown et al., 2014),  
383 but attempts to incorporate this epistasis for the prediction of heterotic traits using  
384 genomic data have been disappointing when the prediction and training set did not  
385 share the same or closely related parents (Jiang and Reif, 2015). This was true even  
386 when using recently developed, efficient models (Jarquín et al., 2014, Martini et al.,  
387 2016) and suggests that genomic data capture only statistical epistasis, referring to  
388 genetic variation at the population level (Sackton and Hartl, 2016), which is gener-  
389 ally of negligible magnitude (Hill, Goddard and Visscher, 2008, Mackay, 2014, Guo  
390 et al., 2016, Vazquez et al., 2016).

## 391 **Complementation of predictors**

392 **Flow of biological information** It is well-known that genetic effects on the phe-  
393 notype are mediated through multiple layers of endophenotypes (Civelek and Luskis,  
394 2014, Ritchie, Holzinger, Li, Pendergrass and Kim, 2015) with information mainly  
395 flowing from the genome toward the phenotype via the transcriptome, the proteome  
396 and the metabolome with metabolite fluxes ultimately governing energy production  
397 and growth (Fiévet, Dillmann and de Vienne, 2010). For most traits in our material,  
398 metabolite- and pedigree-based predictive abilities were lower than those obtained  
399 with either transcriptomic or genomic information. However, consistently high pre-  
400 dictive abilities across multiple traits could be realized when combining multiple  
401 predictors, as has been reported previously in humans (Vazquez et al., 2016) and  
402 maize inbred lines (Guo et al., 2016). This suggests complementary properties of the  
403 different predictors resulting in better proxies for the complex interplay in gene net-  
404 works than genomic information alone. Such an advantage is particularly important  
405 for hybrid prediction when parents of prediction set hybrids are not closely related  
406 to parents of training set hybrids (File S1) as was shown by the relative excellence  
407 of transcriptomic data and the use of multiple predictors for the prediction of traits  
408 in T0 hybrids compared to T1 and T2 hybrids.

409 **Tapping new sources of information** Whereas pedigree and genomic informa-  
410 tion are static, subsequent endophenotypes are characterized by pervasive interac-  
411 tions among and between each other (Dalchau et al., 2011, Zhu et al., 2012) and are,  
412 to varying degrees, influenced by biotic (Rudd et al., 2015, Tzin et al., 2015) and abi-



413 otic perturbations (Caldana et al., 2011, Witt et al., 2012). So while endophenotypes  
414 do not exclusively report on physiological epistasis but also on non-heritable effects,  
415 they seem to capture important information not represented by the genome given  
416 their intermediate position in the genotype-phenotype cascade. We get support for  
417 this hypothesis from (i) merely low to moderate correlations between off-diagonal  
418 elements of the kernels of different predictors in our study, (ii) mounting evidence for  
419 further improvements of predictive abilities when complementing genomic prediction  
420 with other endophenotypes despite sufficient marker densities (Fig. S6, Guo et al.  
421 (2016)) and (iii) the integration of SNP information from close and distant eQTL  
422 in the transcripts analyzed in our study. However, we concede that the number  
423 of parental genotypes in our mRNA assays was too small to warrant a reasonable  
424 statistical power for detecting epistasis in the expression of transcripts. In breed-  
425 ing programs, predictive abilities are largely driven by relationships — including  
426 Mendelian sampling — among genotypes compared to LD between SNP markers  
427 and causal QTL (Schopp et al., 2017). Increasing marker densities therefore have  
428 limited utility for improving genomic predictions as observed in our material, where  
429 SNP-based predictive abilities reached a plateau after using 5,000 equally spaced  
430 SNPs (Fig. S6). While two other studies also attempted to model interactions be-  
431 tween different predictors, we refrained from this approach given that their reported  
432 predictive abilities based on interactions were not different from those in additive  
433 models despite using much larger sample sizes (Vazquez et al., 2016, Guo et al.,  
434 2016).

## 435 **Transcriptomic data**

436 **Utility of transcriptomic data for trait predictions** Of particular note was  
437 the excellent performance of transcriptomic data in predicting dry matter yield and  
438 protein. Evidence that parental gene expression patterns might be predictive of  
439 hybrid performance is given by (i) prevailing additive expression patterns in maize  
440 hybrids (Springer and Stupar, 2007*a*, Stupar et al., 2008), (ii) a positive correlation of  
441 the proportion of additive gene expression with the yield of hybrids (Guo et al., 2006),  
442 and (iii) co-localization of additively expressed genes with heterotic QTL (Thiemann  
443 et al., 2014). According to metabolic flux theory, gene expression in hybrids at the  
444 mid-parent level can generate hybrid vigor by counterbalancing opposing detrimental  
445 expression levels in their parent lines on a genome-wide scale (Kacser and Burns,  
446 1981, Springer and Stupar, 2007*b*). The same concept is expected to apply to other  
447 quantitative endophenotypes (Lisec et al., 2011).

448 **Pre-selection bias** As pointed out earlier, our transcripts were pre-selected based  
449 on associations with grain dry matter yield and grain dry matter content in hybrids,  
450 using a subset of the data included in our study ( $H_{\text{FAC}(1)}$ ). Hence, genotypes used  
451 for the pre-selection (*i.e.*  $H_{\text{FAC}(1)}$ ) could be regarded as a training set. By combining  
452 this "training set" and genotypes from the remaining five factorials, we might have  
453 introduced a bias by using predictors that have already seen the response variable  
454 in the  $H_{\text{FAC}(1)}$  genotypes. To rule out the existence of such a bias, we have com-  
455 pared the predictive abilities of different predictors for the complement of  $H_{\text{FAC}(1)}$ .  
456 Two findings indicate that no bias in the comparison of predictive abilities was in-

457 introduced: (i) Relative differences in predictive abilities between transcriptomic and  
458 pedigree or genomic data did not change when excluding genotypes from  $H_{\text{FAC}^{(1)}}$   
459 from the data (Fig. S7) and (ii) transcriptomic data performed rather poorly in  
460 predicting dry matter content although this trait was also among the criteria for the  
461 pre-selection procedure. Finally, an independent study using RNA-Seq data for the  
462 prediction of traits in maize inbred lines also reported exceptionally good perfor-  
463 mance of transcriptomic data in the prediction of multiple yield-related traits (Guo  
464 et al., 2016).

## 465 **Relative excellence of predictors for different traits**

466 **Tissue and sampling time** Despite the great prospects of using endophenotypes  
467 for trait predictions, some aspects require careful consideration when using this ap-  
468 proach. A particular challenge in endophenotype-based prediction efforts is the choice  
469 of a suitable tissue and sampling time. Tissue-related effects regarding gene expres-  
470 sion were found in studies on humans (Yang et al., 2015, Mele et al., 2015, Searle  
471 et al., 2016) and *A. thaliana* (Schmid et al., 2005) and in maize hybrids with respect  
472 to metabolome composition and metabolite abundance (Witt et al., 2012). Moreover,  
473 the age of an organism can selectively influence the expression of genes as observed in  
474 studies on humans (Mele et al., 2015, Yang et al., 2015) and *C. elegans* (Vinuela et al.,  
475 2010, Francesconi and Lehner, 2014). The low correlations between the off-diagonal  
476 elements of the kernels calculated from root and leaf metabolites might therefore  
477 be a reflection of highly dynamic processes differing between tissues and during  
478 different developmental stages. Whereas root metabolic data and transcriptomic

479 data were obtained from seedlings germinated in standard controlled conditions, leaf  
480 metabolic data were derived from field-grown plants at a much later developmental  
481 stage, thereby increasing the possibility of environmentally-induced modifications.  
482 One might hypothesize, that the choice of sampling time and tissue could influence  
483 the chances of successful trait prediction if such age- or tissue-dependent transcripts  
484 and metabolites are associated with a phenotypic or clinical trait.

485 **Feature selection** Another explanation for trait-dependent excellence of any pre-  
486 dictor might lie in the sampling of features. In this study, only a small subset of  
487 metabolites was sampled and even very recent technologies (Xu et al., 2016, Dan  
488 et al., 2016) capture only a fraction of the estimated set of metabolites (Fernie,  
489 2007). Moreover, the smaller differences in metabolite levels between both heterotic  
490 groups (Fig. 1) were most likely not conducive to capturing basic components un-  
491 derlying complex heterotic traits. It is also possible that transcriptomic data are  
492 associated with more biological processes than metabolite data and better capture  
493 the genetic effects relevant for the prediction of T0 hybrids.

494 **Prospects for metabolites** Previously observed moderate metabolite-based pre-  
495 dictive abilities for T1 hybrids (Riedelsheimer et al., 2012) were confirmed in our  
496 study (Fig. S8), but for the majority of traits, root metabolites reached only medium  
497 and leaf metabolites even lower predictive abilities when predicting T0 hybrids. De-  
498 spite the aforementioned shortcomings of metabolites, they have shown to be intrigu-  
499 ing predictors due to their physiological proximity to the phenotype, which provides  
500 information that is impossible to infer from DNA or proteins (Fernie and Stitt, 2012),

501 as well as encouraging results from other studies (Guo et al., 2016, Dan et al., 2016).  
502 A recently introduced technology, allowing for live-measurements of small molecules  
503 in the blood of living and awake animals (Arroyo-Currás et al., 2017), might overcome  
504 the problem of poorly time-resolved snap-shots of some metabolites with extremely  
505 fast turnover rates (Arrivault et al., 2009) if modified to properly work in plants.

506 **Predictor requirements** Besides improving upon predictions based on pedigree  
507 relationships by capturing Mendelian sampling, the widespread use of genomic in-  
508 formation in trait prediction has been driven by the ease of its application. In  
509 order to compete with genomic data, other 'omics' data therefore require the use  
510 of standardized sampling conditions to obtain large repeatabilities and the possibil-  
511 ity of season-independent sample extraction from seeds, seedlings or young roots to  
512 achieve high throughput.

## 513 **Conclusions**

514 The use of whole-genome information has considerably advanced trait prediction over  
515 traditional pedigree-based BLUP by incorporating previously unobservable Mendelian  
516 sampling. Combining variegated sources of information promises to capture complex  
517 interactions between genes and endophenotypes, leading to stable predictions across  
518 traits. Especially if an extremely small fraction of the candidates is selected from the  
519 millions of possible new hybrids from each breeding cycle, the success of forecasts  
520 is a strongly convex function of predictive ability (Fig. 3b). Therefore, consider-  
521 ing endophenotypes could have a substantial effect on the success and economics

522 of hybrid breeding. Given the anticipated technological improvements in RNA-Seq  
523 and metabolite profiling, as well as the forthcoming adoption of the DH-technology  
524 for many crops (Kelliher et al., 2017), a paradigm shift from exclusively genomic  
525 prediction models to more inclusive approaches seems imminent.

## Author Contributions

W.S. and A.E.M developed the lines and hybrids, W.S. and A.E.M designed the field experiments, T.A.S analyzed the agronomic and pedigree data, L.W., M.S., A.S., A.E.M and M.W. designed the metabolic experiments, A.S. conducted the metabolic experiments, S.S. designed the transcriptomic experiments, F.S. and A.T. conducted the transcriptomic experiments, M.W. analyzed the metabolic and transcriptomic data, M.W., T.A.S, G.T., C.H. and A.E.M. devised the prediction models, M.W., C.H. and T.A.S. implemented the prediction models and developed software, H.F.U. and A.E. contributed to the statistical analysis. M.W., A.E.M., S.S, G.T., Z.N. and C.C.S. wrote the manuscript.

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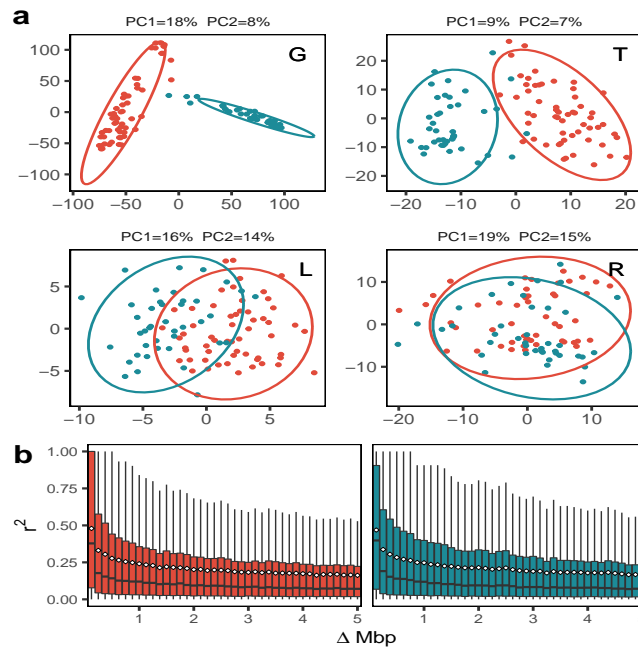


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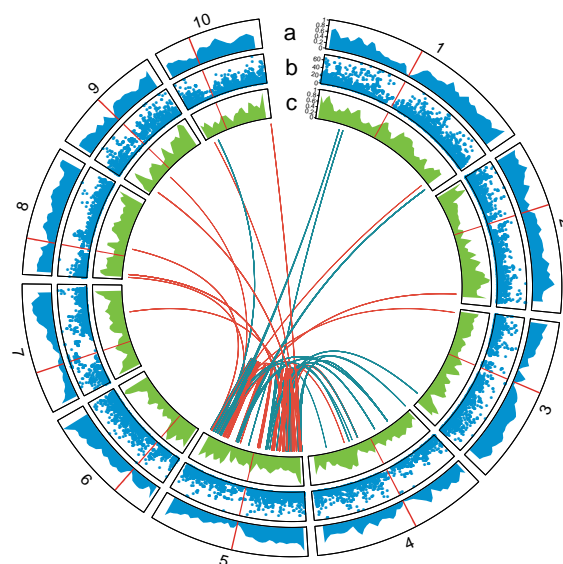


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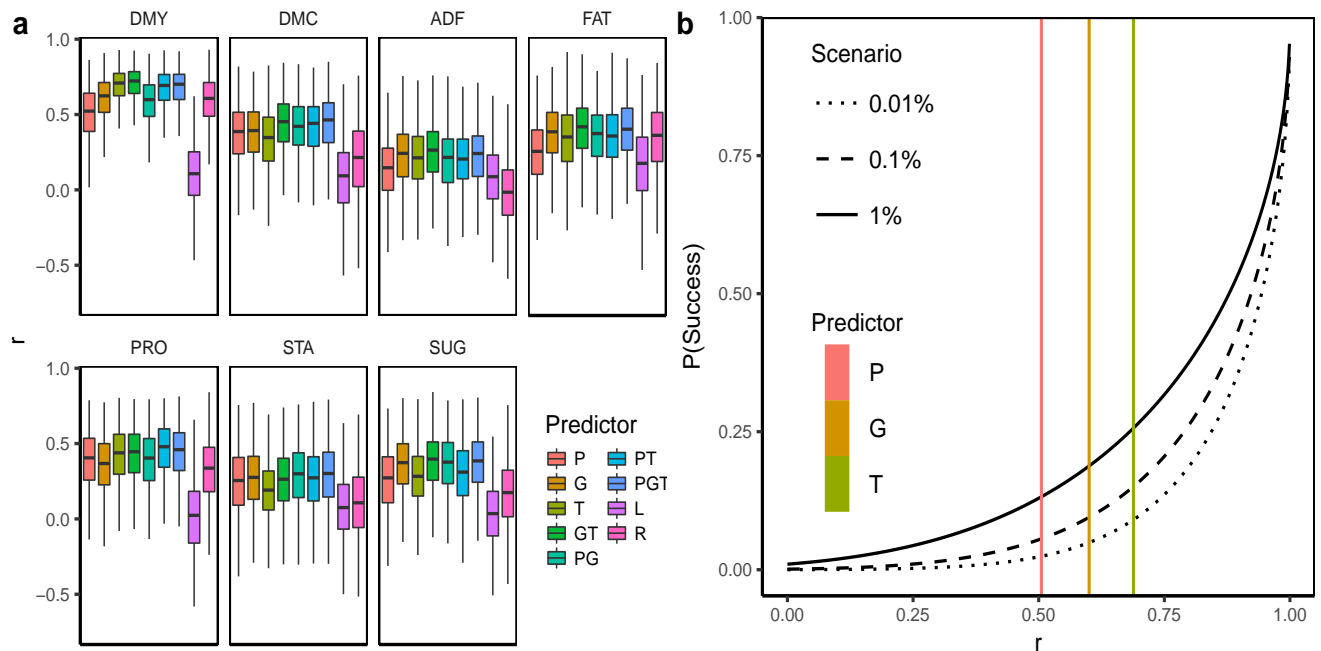


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Trait	$\mu$	$\sigma_{GCA^D}^2$	$\sigma_{GCA^F}^2$	$\sigma_{SCA}^2$	$H^2$
DMY (t/ha)	19.00	1.51 $\pm$ 0.22	1.00 $\pm$ 0.18	0.17 $\pm$ 0.03	0.82
DMC (%)	34.13	4.17 $\pm$ 0.59	5.03 $\pm$ 0.79	0.49 $\pm$ 0.07	0.91
ADF (%)	20.93	0.27 $\pm$ 0.06	0.40 $\pm$ 0.09	0.02 $\pm$ 0.02	0.43
FAT ( $\%_0$ )	30.02	1.01 $\pm$ 0.19	2.10 $\pm$ 0.37	0.17 $\pm$ 0.05	0.73
PRO ( $\%_0$ )	69.65	3.11 $\pm$ 0.53	2.77 $\pm$ 0.51	0.29 $\pm$ 0.10	0.70
STA (%)	35.56	2.95 $\pm$ 0.51	3.33 $\pm$ 0.63	0.24 $\pm$ 0.10	0.69
SUG ( $\%_0$ )	38.12	31.33 $\pm$ 5.15	31.90 $\pm$ 5.73	4.12 $\pm$ 1.02	0.77