

Comprehensive Identification of Genomic and Environmental Determinants of Phenotypic

Plasticity in Maize

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Supplemental Materials

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43 Supplemental_Code_File_1.R)

45

Supplemental Methods

46 Assessment of Impacts of Environment Number on Accuracy of CERIS

47 Recent work systematically evaluated the number of environments needed to accurately
48 estimate and predict slope and intercept with empirical data from two crops (Guo et al. 2024). These
49 results showed that it is not only the number of environments observed but also the range of the
50 environmental mean that they cover that contributes to the accuracy of reaction norm parameter estimates
51 (Guo et al. 2024). From sampling subsets of a total of 9 environments, a plateau in accuracy was
52 consistently observed in both intercept and slope estimates in both crops when the subset included at least
53 4 environments and/or covered an environmental mean range at least 25-50% of the minimum
54 environmental mean observed (Guo et al. 2024). As few as two environments could provide good
55 estimates, as long as they covered a wide environmental gradient.

56 To check for impacts of number of observed environments on prediction accuracies, we
57 examined the correlation between these and found a significant positive correlation (Fig. S7A: $r = 0.47, P < 0.001$). However, those traits regarded as important and requiring less additional work beyond the field
59 observations were measured in more environments (e.g., $n=11$ environments for flowering traits DTA and
60 DTS, considered less complex to predict) than were yield traits that require harvesting and processing for
61 measurements (e.g., $n=5$ environments for yield traits T20KW and KN, considered more complex to
62 predict) (Table S1) (Onogi 2022; Li et al. 2021). To differentiate the relative importance of trait type and
63 environment number to the overall trend, we checked the correlation within trait type, and found that
64 correlations decreased notably (Fig. S7B, $r = 0.26-0.30, P < 0.001$), indicating that much of the overall
65 trend came from the connection of trait type (and prediction complexity) with observation number.

66 The range of measured environment number was 5-11 for all traits and an environmental mean
67 range of at least 25% of the minimum environmental mean was observed for all but 4 traits (ERN, CD,
68 LL, and T20KW), indicating that our data likely reached the plateau found by (Guo et al. 2024) based on
69 the number of environments and therefore should provide accurate estimates of slope and intercept,

70 though of course there may be room for further improvement, particularly for the traits for which a lower
71 environmental range was covered.

72

73 **Construction of Candidate Gene List**

74 The full candidate gene list (Table S4) was constructed by building on previous work in the
75 NAM panel that defined candidate genes as those within a 20kb window centered on each significant
76 marker from GWAS (Kusmec et al. 2017). Each significant marker has segregating (bi-allelic) variants in
77 the NAM and was found to be associated with trait variation using GWAS. In general, choosing an
78 appropriate window for candidate gene identification is always a challenging topic and a subject of future
79 research. Therefore, we checked 13 different window sizes (4kb, 10kb, 20kb, 30kb, 40kb, 60kb, 80kb,
80 100kb, 150kb, 200kb, and 250kb) to confirm consistency of general patterns (Fig. S10). The chosen 20kb
81 window corresponds to an average LD (r^2) of 0.16 in our data, which is within the typical r^2 range of 0.1 –
82 0.2 for delineating candidate regions (Vos et al. 2017). To enable readers to investigate other windows,
83 we also made all significant SNPs available in Table S3 as well as at MaizeGDB.

84 The candidate gene list was based on the current B73 genome assembly (Zm-B73-
85 REFERENCE-NAM-5.0) for several reasons. First, in the maize NAM population, approximately 50% of
86 the genetic material of a given RIL originates from B73 and the other 50% from another NAM parent. In
87 the population as a whole, therefore, 50% of the genetic material originates from B73, while only ~1.9%
88 originates from each of the other parents (Yu et al. 2008), providing substantially less confidence in any
89 inferences about non-core candidate genes sourced from other (non-B73) NAM parents vs. those found in
90 B73. In addition, the recent re-sequencing of the NAM founders, which generated the high-density SNP
91 and SV marker data utilized here, mapped all markers to the B73 genome (Hufford et al. 2021). It was
92 cleaner to keep the annotations consistent with the genomic coordinates used to identify these SNPs and
93 SVs. Finally, because by definition core genes are present in all annotations, our method did not exclude
94 any core genes. Therefore, we focused on B73 genes in our investigation. In doing so, we also follow

95 established precedent for GWAS in this population (Hufford et al. 2021) and for other cases with pan-
96 genomes available (Della Coletta et al. 2021).

97 Based on the >20 million SNP and SV markers used for GWAS, 94% of the candidate genes
98 identified had at least one SNP or SV marker within the gene itself, increasing to >99% when the search
99 was broadened to include 5kb upstream. Among the remaining genes, manual examination revealed non-
100 marker polymorphisms; for example, Zm00001eb036690 had no markers within it, but BRIDGEcereal
101 identified large indels within this gene among the NAM founders. Because of the presence of non-marker
102 polymorphisms as well as our goal to provide a community resource with all significant results available
103 for ongoing investigation, we chose to retain the remaining <1% of genes from our candidate gene list.

104

105 **BRIDGEcereal Haplotype Visualization**

106 BRIDGEcereal (<https://bridgecereal.scinet.usda.gov/>) (Zhang et al. 2023) was used to manually
107 identify indel-based haplotypes among the NAM parents for a subset of the candidate genes (“selected”
108 candidate genes) in the example trait DTA (Fig. S11, Fig. S12, Fig. S13). For each gene, the 26 parents
109 were grouped into two or three “genotype groups,” representing different BRIDGEcereal-detected
110 haplotypes based on large indels, which were then named after a representative parent in that group. The
111 phenotype estimates for each NAM parent within a given genotype group were also plotted (Fig. S11,
112 Fig. S12, Fig. S13).

113

114 **Supplemental Methods Sources**

115 Della Coletta R, Qiu Y, Ou S, Hufford MB, Hirsch CN. 2021. How the pan-genome is changing
116 crop genomics and improvement. *Genome Biol* **22**: 3.
117 Guo T, Wei J, Li X, Yu J. 2024. Environmental context of phenotypic plasticity in flowering
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119 Hufford MB, Seetharam AS, Woodhouse MR, Chougule KM, Ou S, Liu J, Ricci WA, Guo T,

120 Olson A, Qiu Y, et al. 2021. De novo assembly, annotation, and comparative analysis of 26
121 diverse maize genomes. *Science* **373**: 655–662.

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123 phenotype means and plasticities in *Zea mays*. *Nat Plants* **3**: 715–723.

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128 predictions (GP). In *Genomic Prediction of Complex Traits: Methods and Protocols* (eds.
129 N. Ahmadi and J. Bartholomé), pp. 359–396, Springer US, New York, NY.

130 Vos PG, Paulo MJ, Voorrips RE, Visser RGF, van Eck HJ, van Eeuwijk FA. 2017. Evaluation of
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132 potato. *TAG Theor Appl Genet Theor Angew Genet* **130**: 123–135.

133 Yu J, Holland JB, McMullen MD, Buckler ES. 2008. Genetic Design and Statistical Power of
134 Nested Association Mapping in Maize. *Genetics* **178**: 539–551.

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136 Yu J, Li X. 2023. Streamline unsupervised machine learning to survey and graph indel-
137 based haplotypes from pan-genomes. *Mol Plant* **16**: 975–978.

138

Supplemental Figures

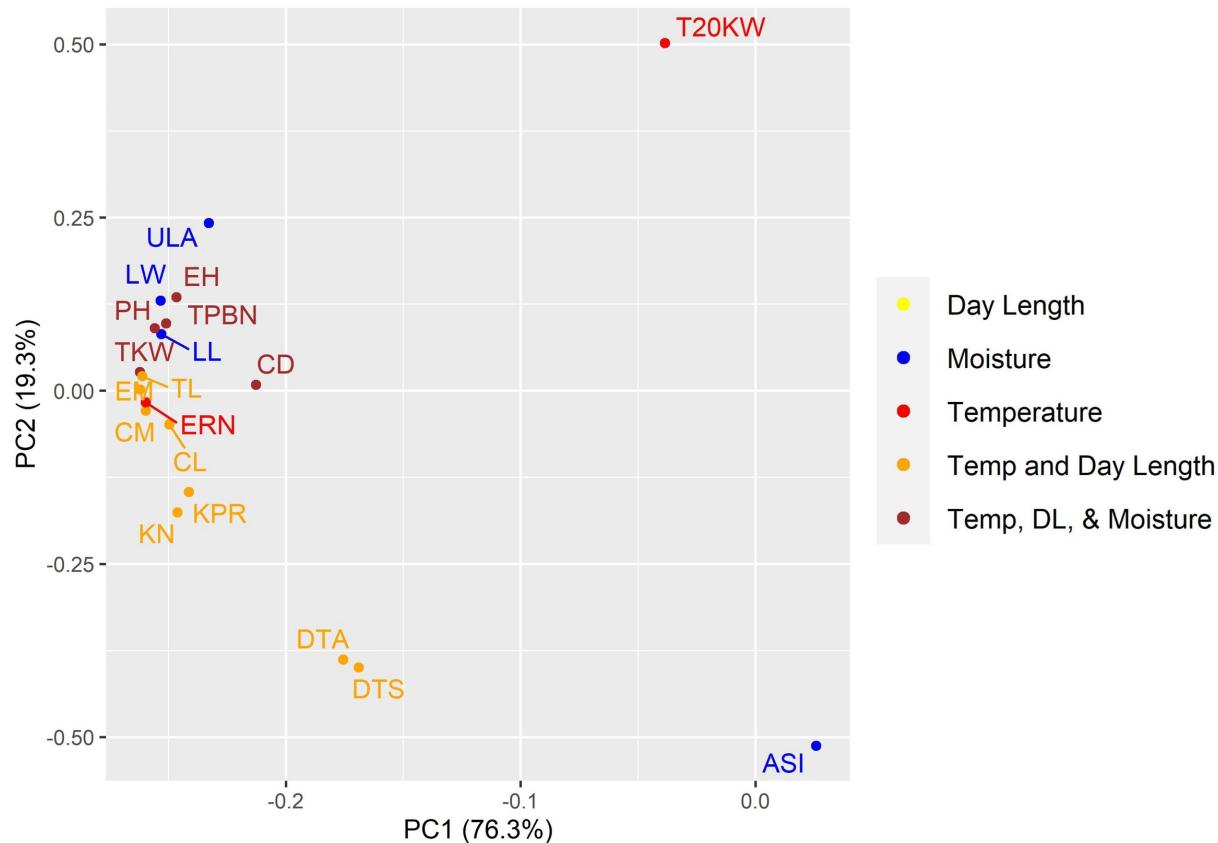
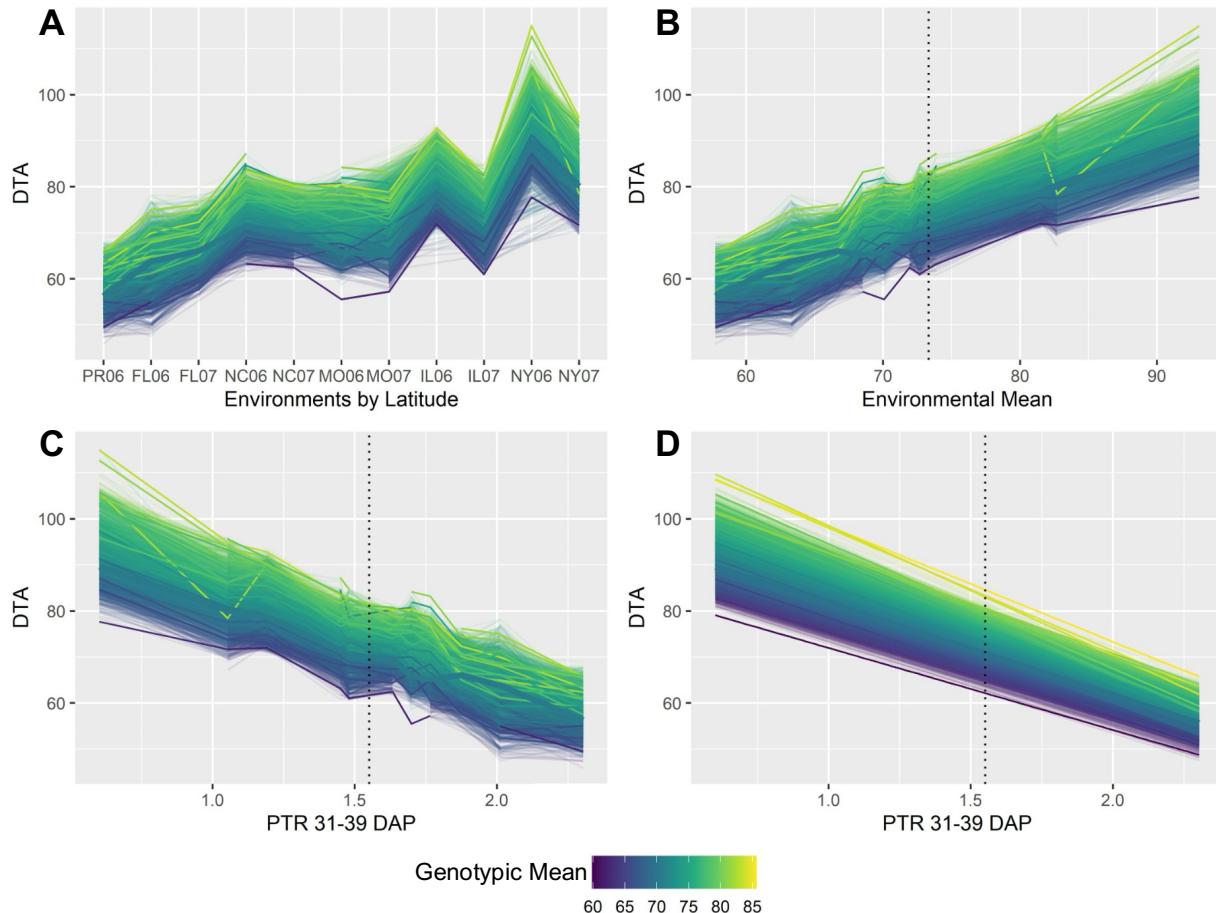


Fig. S1: Principal Components Analysis (PCA) of environmental means reflect environmental indices identified by CERIS. Principal Components (PCs) were calculated based on the scaled and centered environmental mean for each trait; percent of variance explained by each PC is shown on axes. Traits are colored by the environmental variable(s) chosen for them by CERIS.

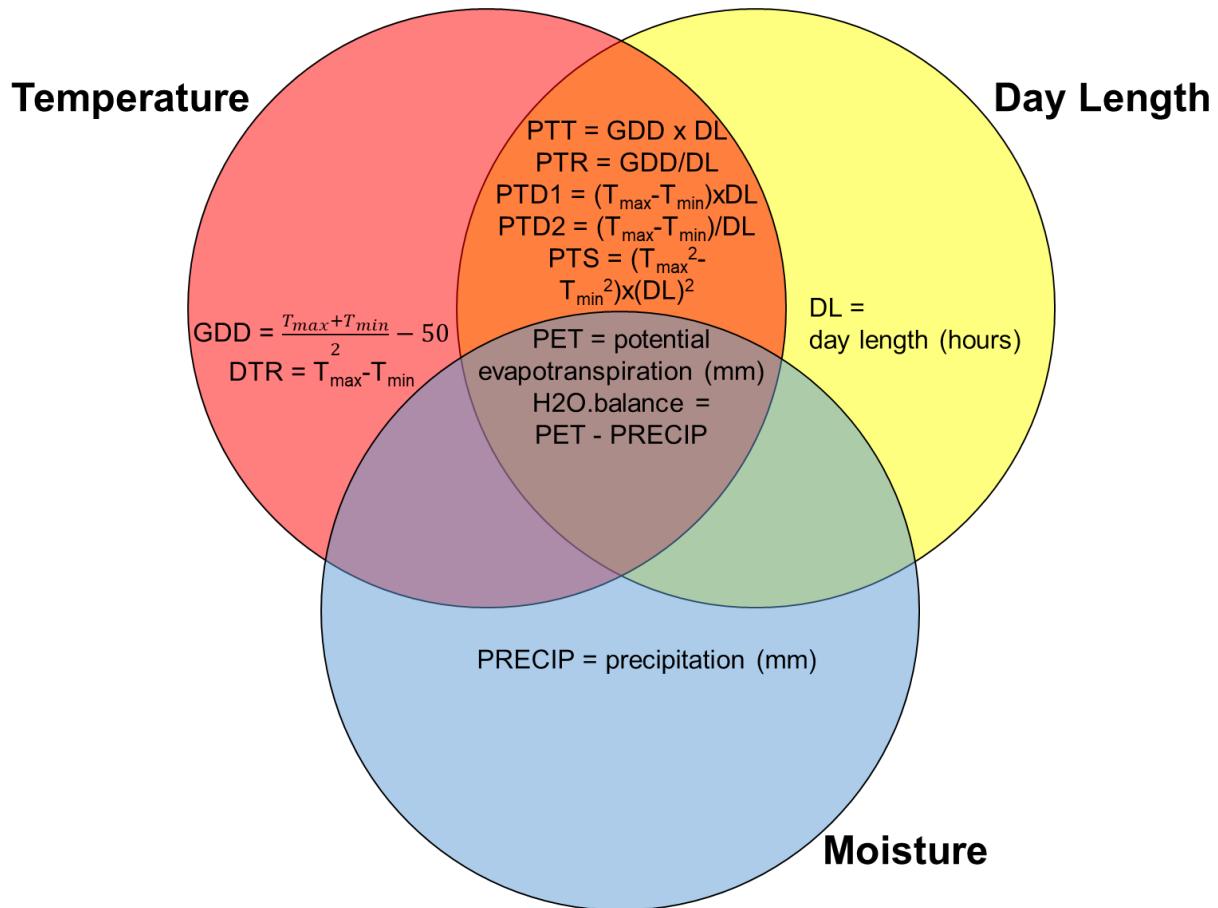
Abbreviations: temperature (temp) and day length (DL).



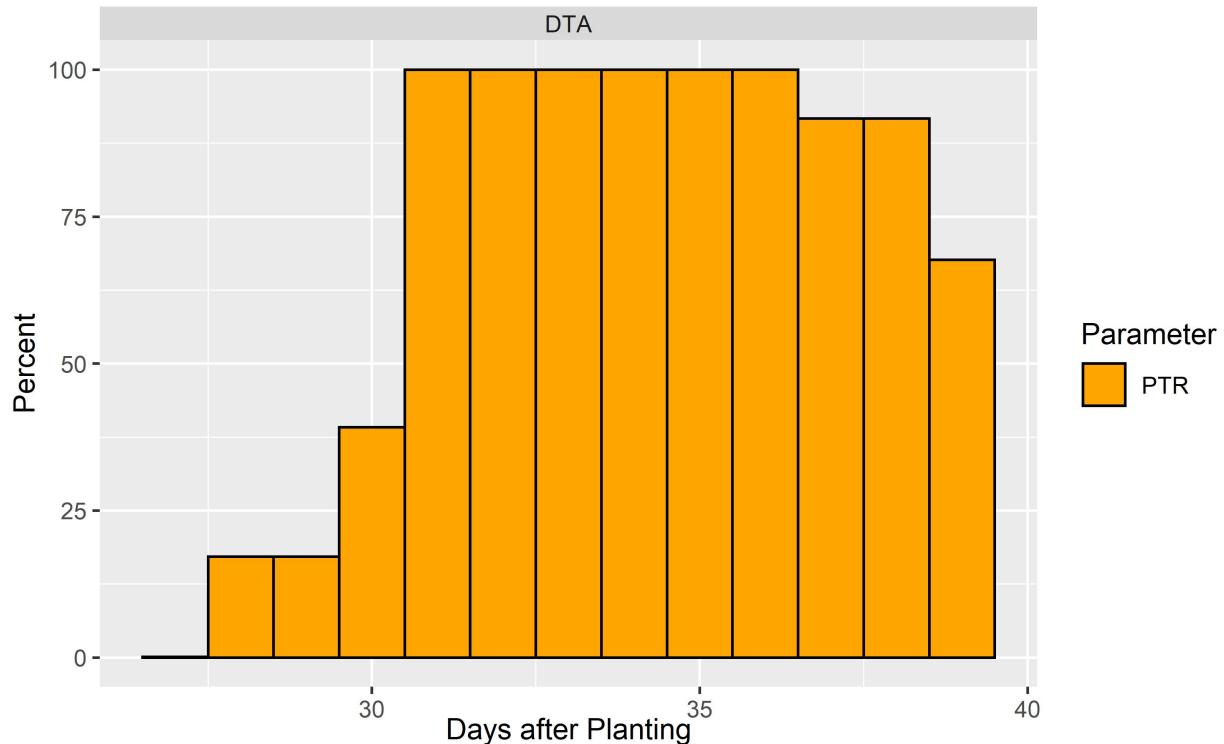
147

148 Fig. S2: Pattern detection for phenotypic plasticity of days to anthesis (DTA) in the maize NAM
 149 population. (A) DTA across environments ordered by latitude. Environments are named as the
 150 two-letter state abbreviation with the last two digits of the year (e.g., MO06 is Missouri 2006).
 151 (B) DTA across environmental mean values. (C) DTA across values of the CERIS-selected
 152 environmental index (PTR 31-39 DAP). (D) Linear reaction norm of DTA across the
 153 environmental index values, calculated using random regression as used in CERIS-JGRA
 154 predictions. In all panels, lines connect DTA values of a given genotype across environments;
 155 line color indicates genotypic mean. The thick line denotes the common parent B73, and other
 156 opaque lines denote other NAM parents.

157



160 Fig. S3: Environmental variables. The environmental variables searched by CERIS include
 161 measures of temperature, day length, moisture, and combinations of these.

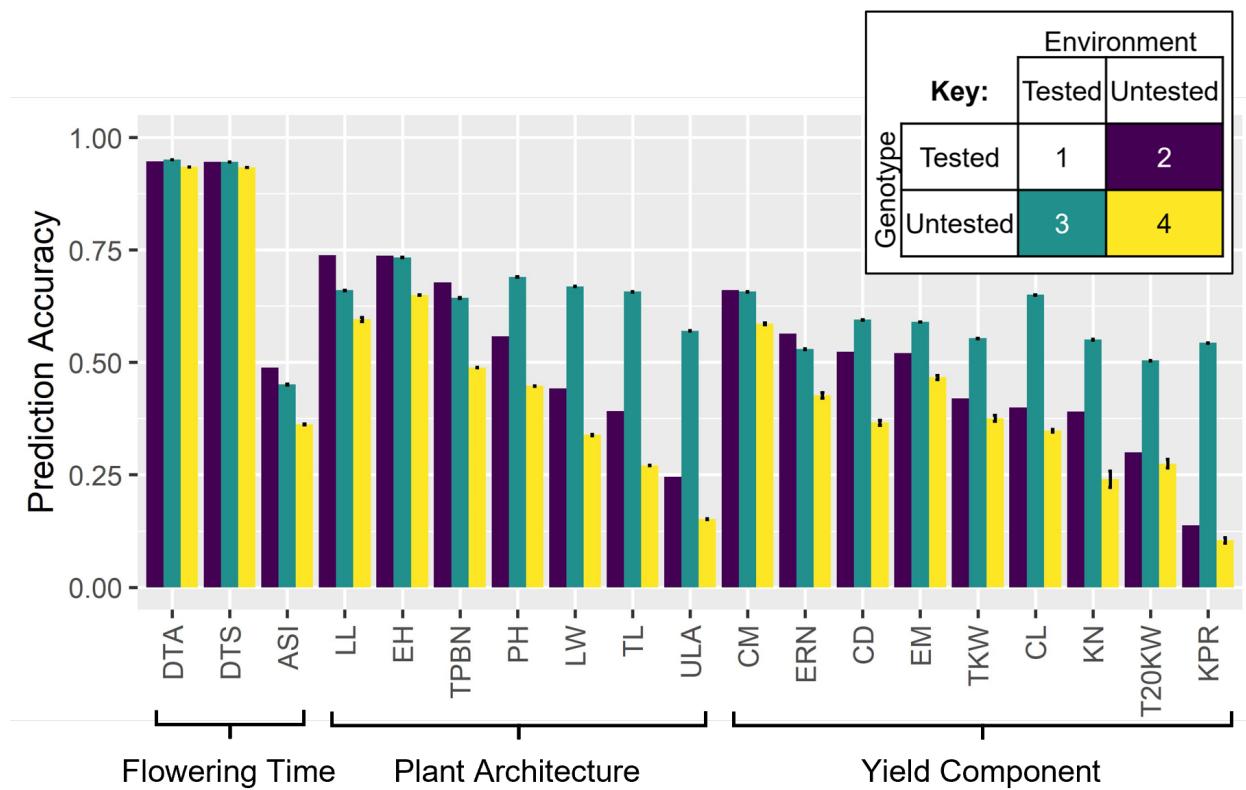


163

164 Fig. S4: CERIS-chosen environmental indices are robust to training set. For the example trait
 165 DTA, CERIS was conducted on more than 700 different training sets (see Methods). This
 166 histogram shows how often a given day after planting was included in the CERIS-chosen
 167 environmental index; color indicates the chosen parameter. 100% of training sets yielded
 168 environmental indices using the environmental variable PTR and including the time range of 31-
 169 36 days after planting.

170

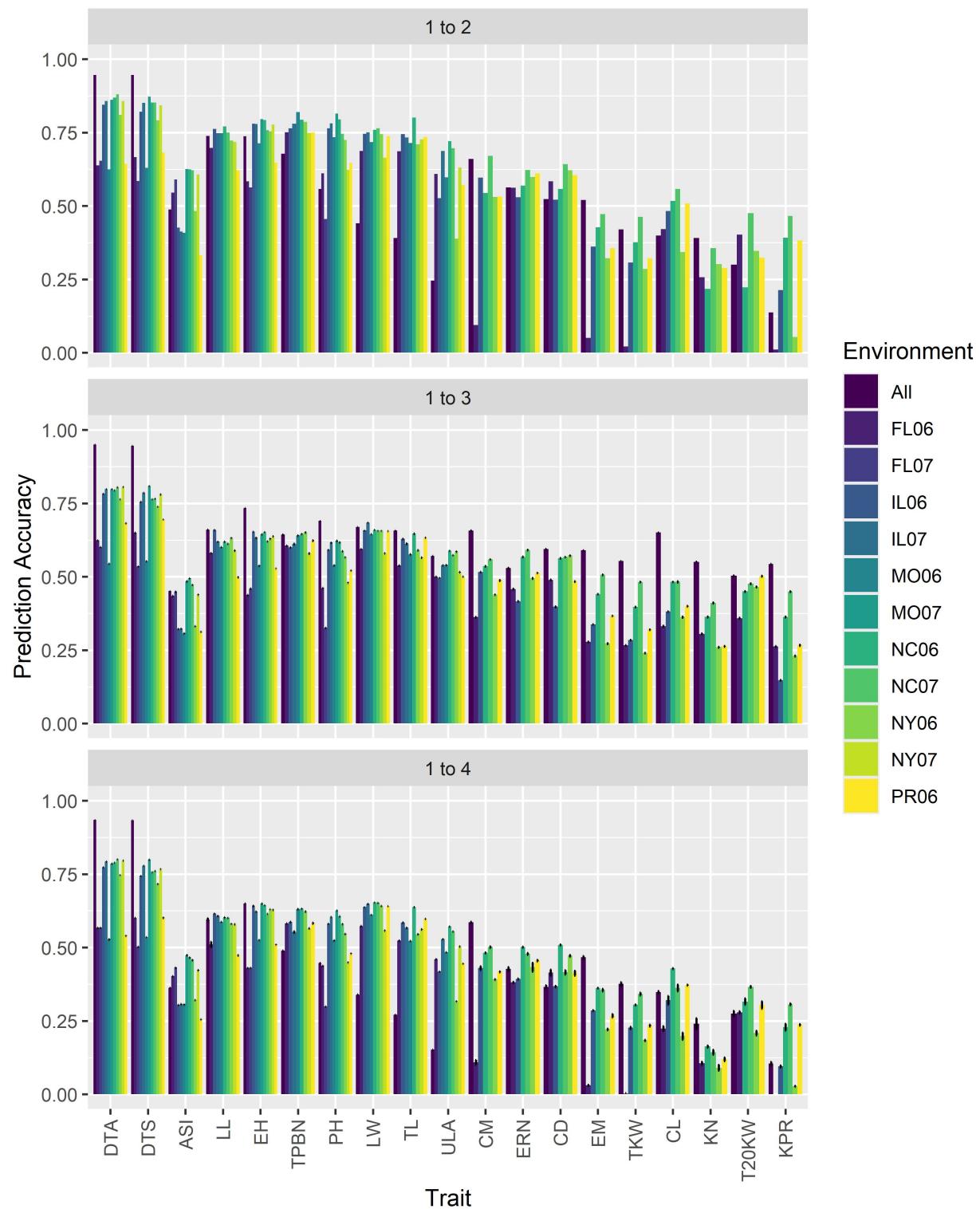
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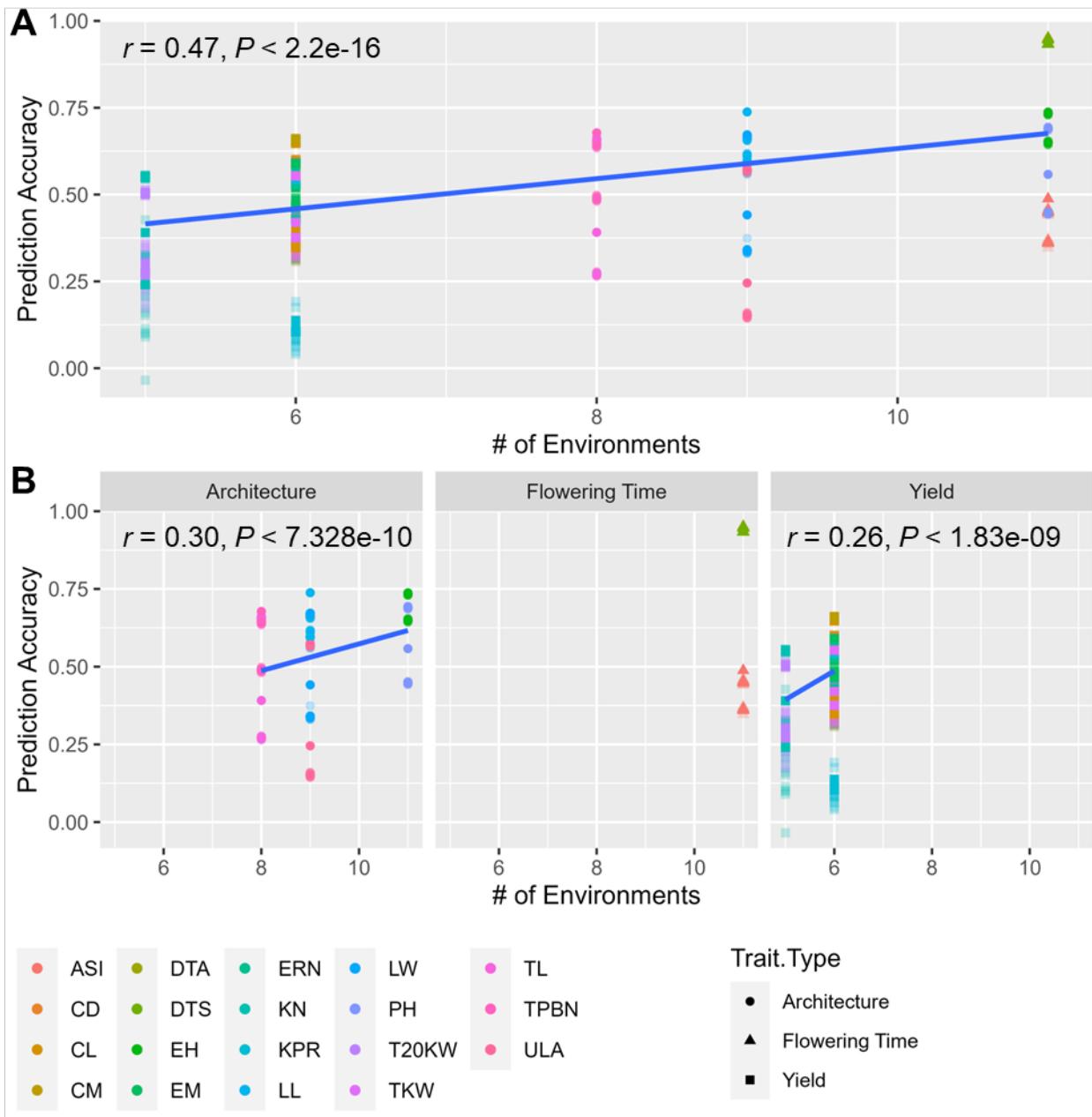
173 Fig. S5: Prediction accuracy for all traits, overall across all environments. Prediction accuracy
 174 for 1 to 2 (purple), 1 to 3 (green), and 1 to 4 (yellow) prediction scenarios for flowering time,
 175 plant architecture, and yield component traits. Key (inset) shows naming scheme for prediction
 176 scenarios; left and right columns denote tested and untested genotypes, respectively, while the
 177 top and bottom rows denote tested and untested genotypes. Error bars show standard error of
 178 prediction accuracy from 30 replicates. Traits ordered by prediction accuracy in the 1 to 2
 179 scenario within each trait group.

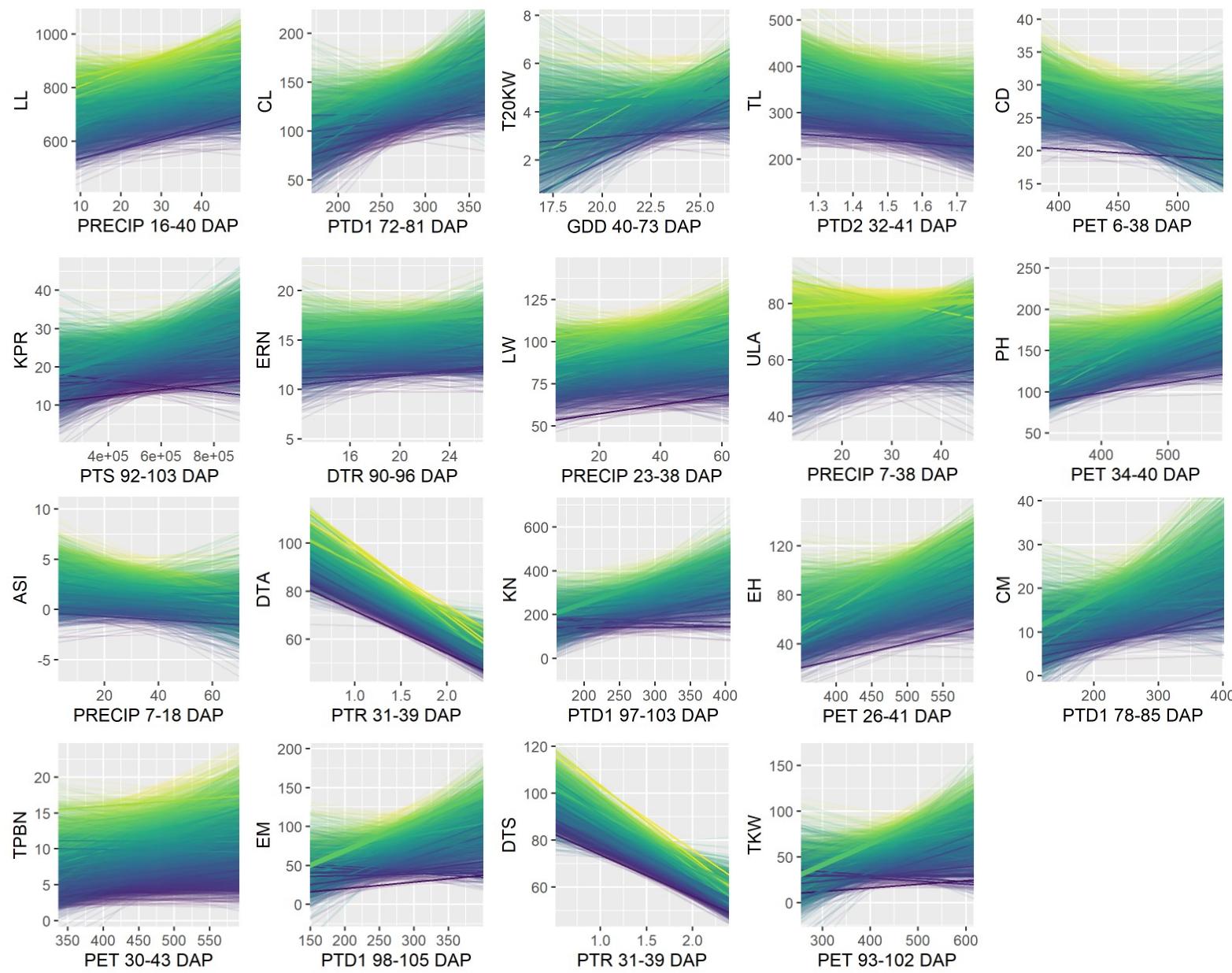
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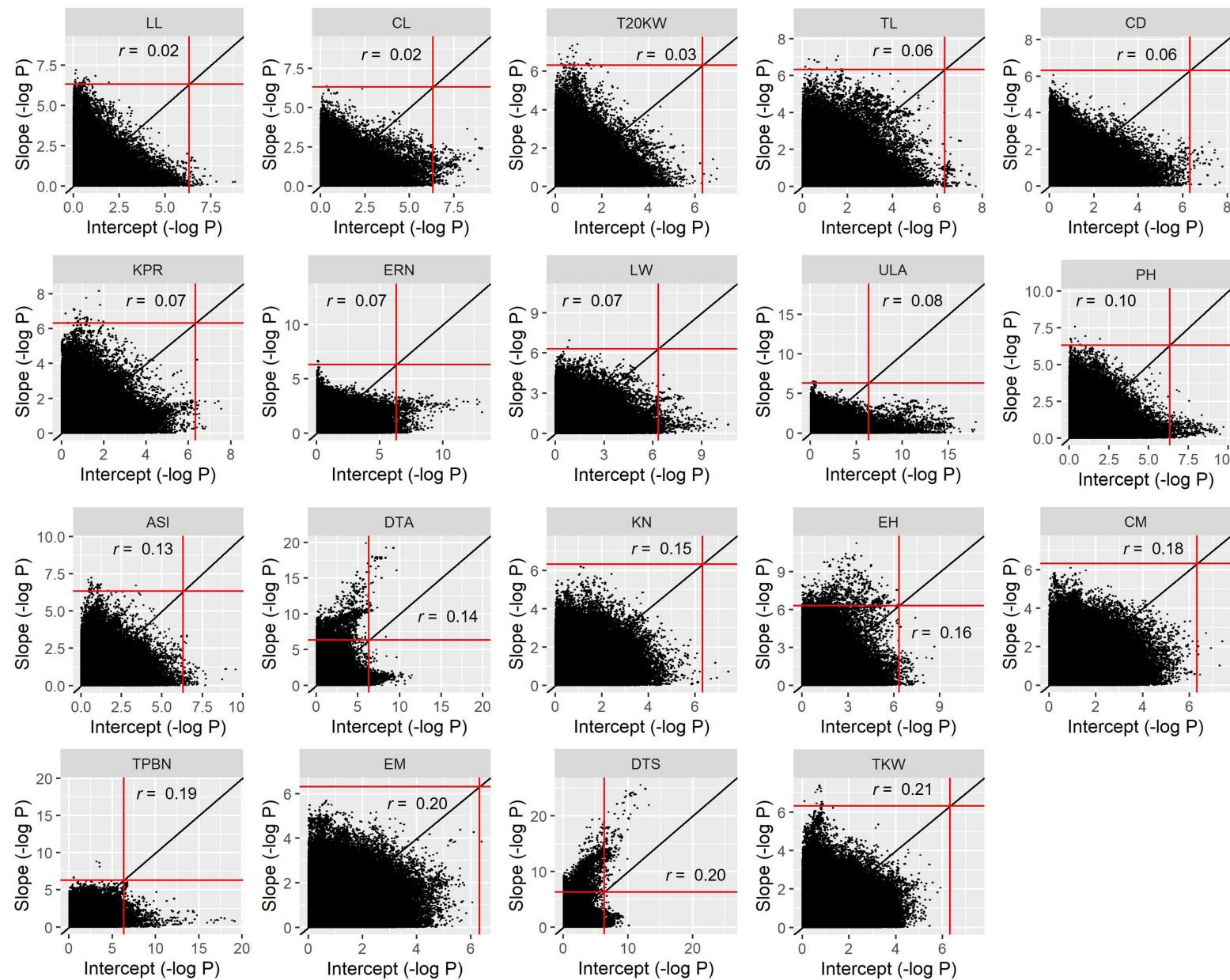
183 Fig. S6: Prediction accuracy for all traits, shown both within and across environments. Prediction
184 accuracy for 1 to 2, 1 to 3, and 1 to 4 prediction scenarios for all traits across environments
185 (“All”) as well as within each measured environment. Error bars show standard error of
186 prediction accuracy from 30 replicates. Trait order corresponds to Fig. S5.

187

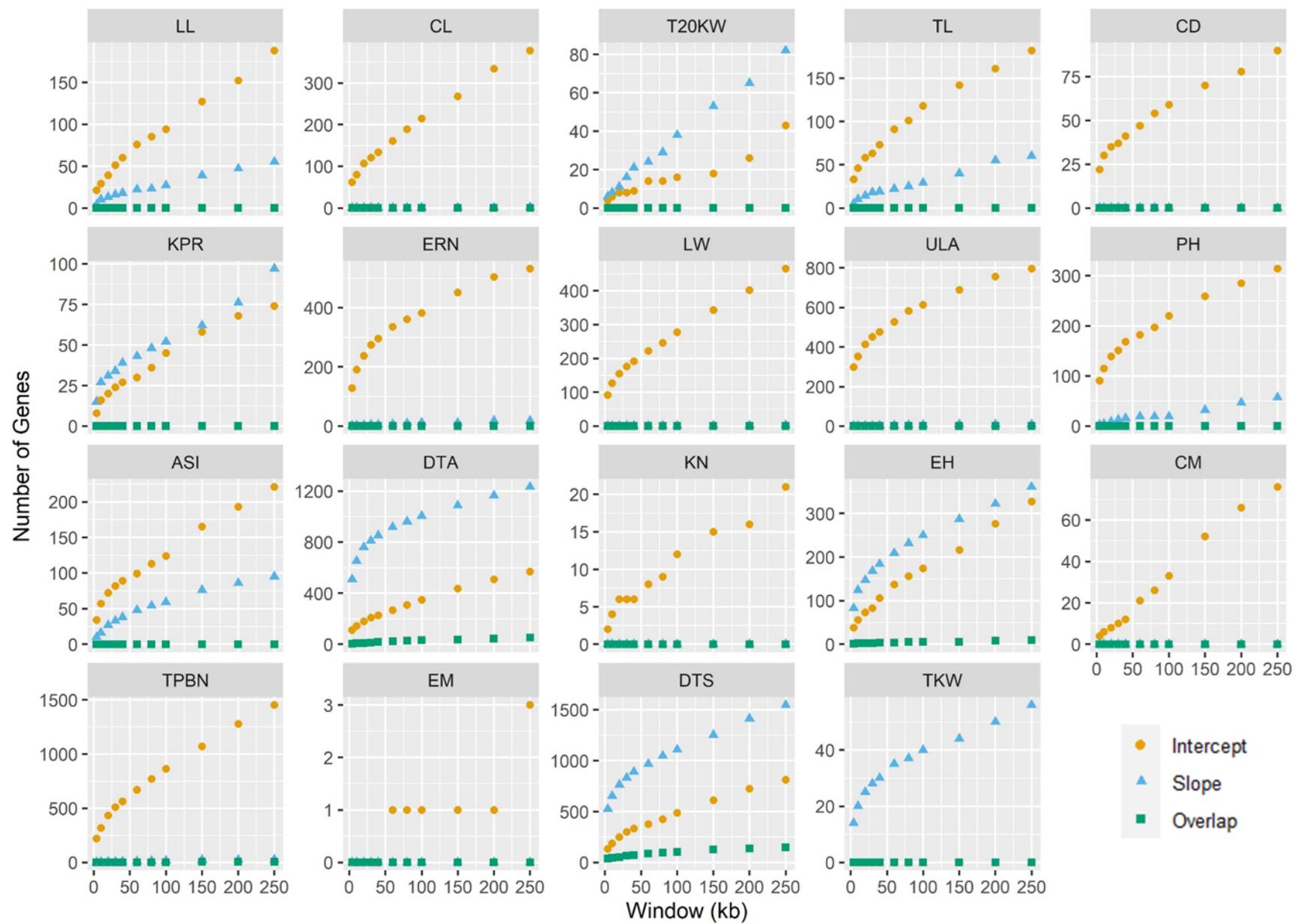




195 Fig. S8: CERIS reaction norms for all traits. In all panels, the thick line denotes the common parent B73 and opaque lines the other
196 parents; line color indicates the genotypic mean, ranked within a given trait. Slope and intercept estimates from these reaction norms
197 using fixed regression were used as input phenotypes in GWAS. Traits are ordered by the correlation between $-\log_{10}(P)$ values from
198 slope and intercept GWAS to show the continuum of genetic architectures of plasticity.

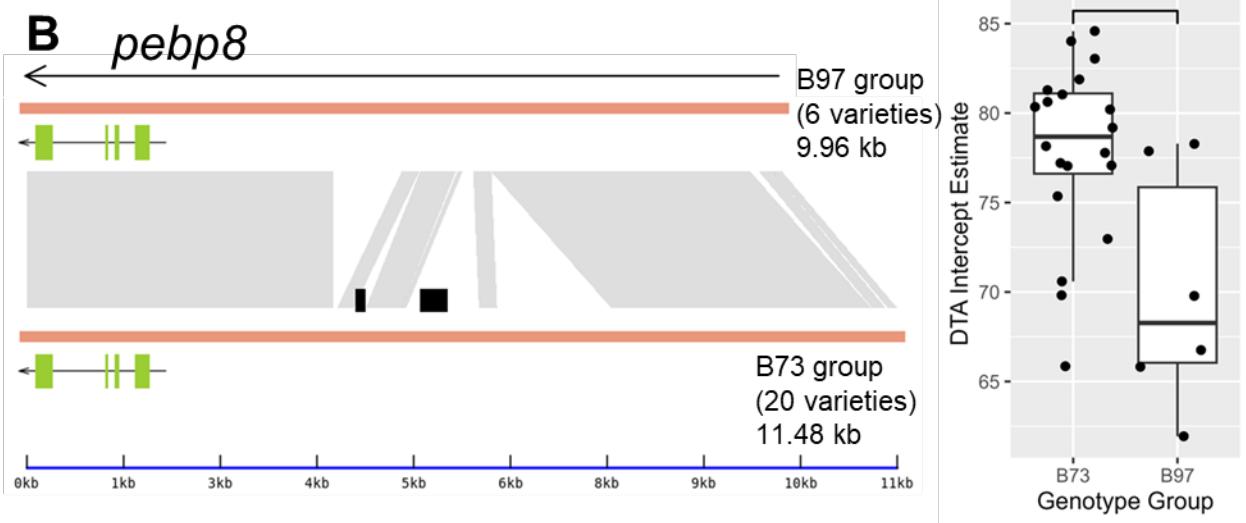
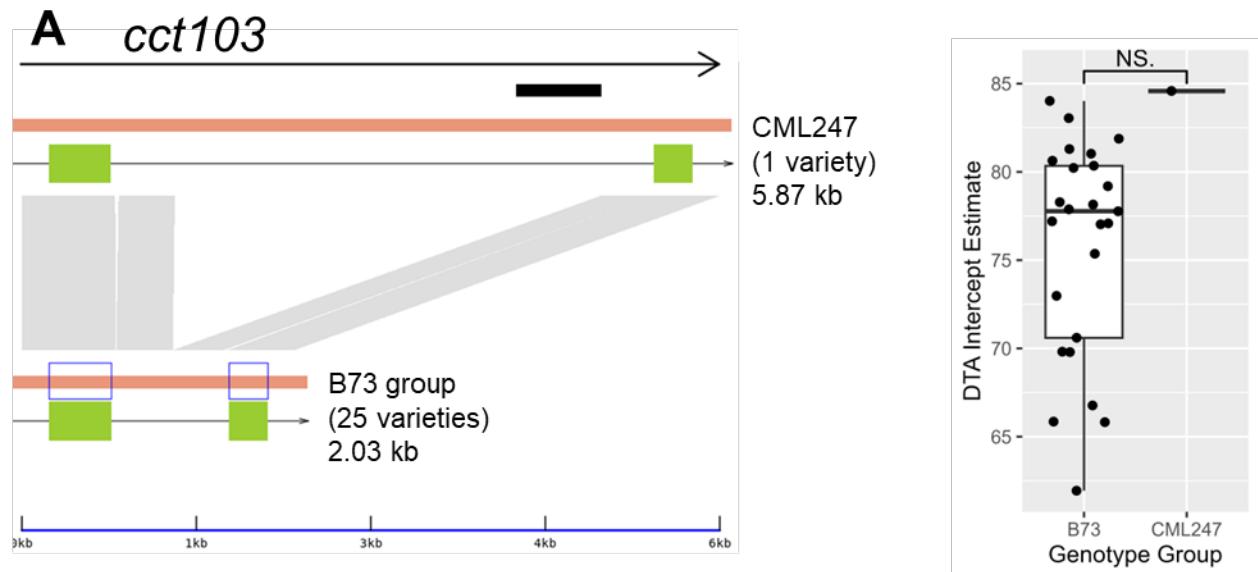


200 Fig. S9: P values of markers from intercept and slope GWAS. For each trait, the $-\log_{10}(P)$ values from slope and intercept GWAS are
201 shown on the y and x axis, respectively. In all cases, these $-\log_{10}(P)$ values were significantly ($P < 0.00001$) positively correlated. Red
202 lines show the SimpleM significance threshold, and the black line indicates the line where $x = y$. Traits ordered by the correlation
203 between $-\log_{10}(P)$ values from slope and intercept GWAS to show the continuum of genetic architectures of plasticity



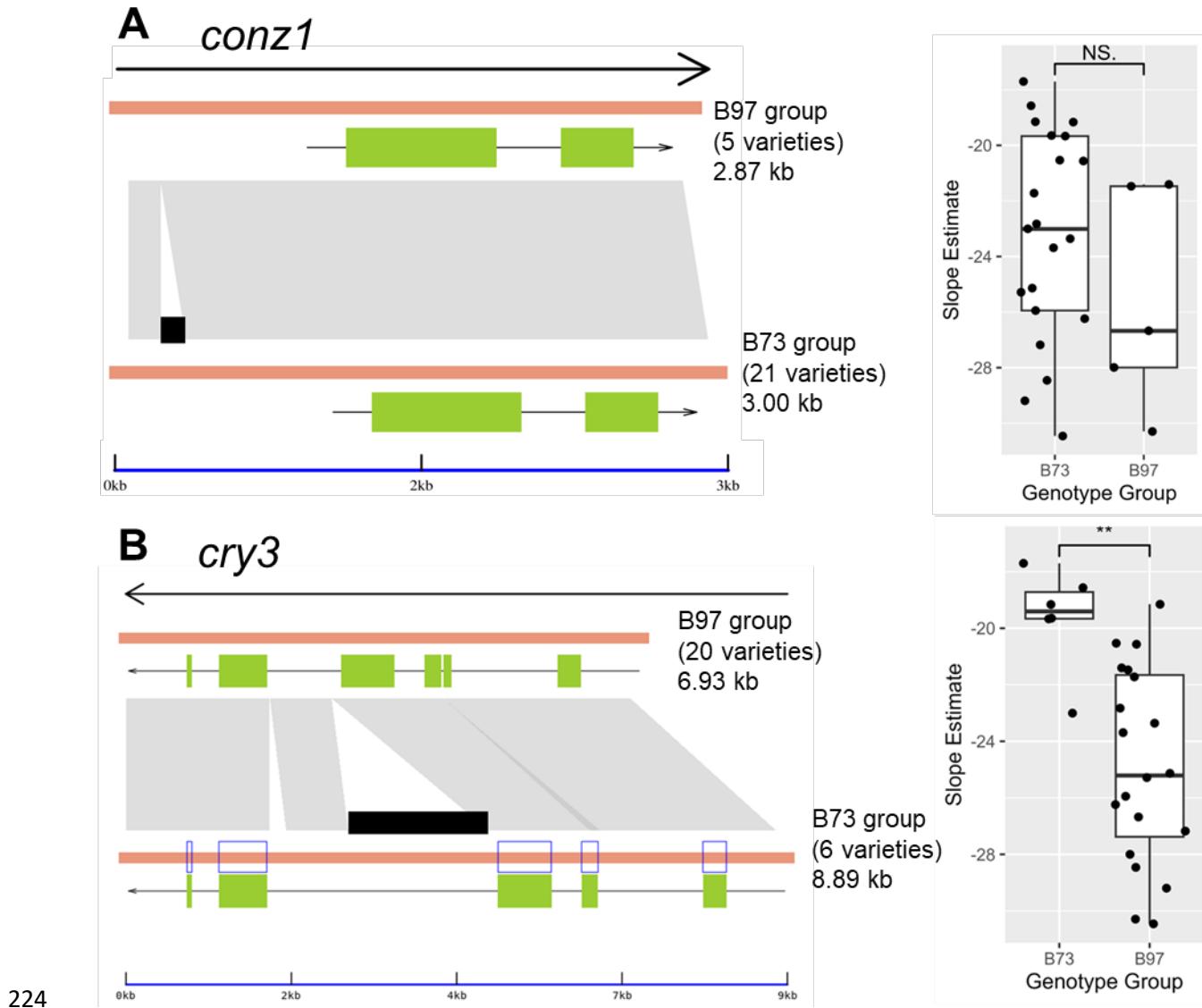
205 Fig. S10: Number of candidate genes by trait for all examined window sizes. Number of genes (y axis) located within a given window
206 (x axis) of significant markers detected in intercept (orange circles) or slope (blue triangles) GWAS as well as those detected in both
207 (“overlap”, green squares). Traits ordered by the correlation between $-\log_{10}(P)$ values from slope and intercept GWAS to show the
208 continuum of genetic architectures of plasticity.

209



212 Fig. S11: BRIDGEcereal visualization of major haplotypes for DTA intercept candidate genes
213 and associated phenotype estimates among the NAM founders. (A) CML247 has both the highest
214 DTA intercept estimate among the NAM founders and a unique haplotype at *cct103*,
215 distinguished by an insertion in the intron. The CML247 genotype group contains only CML247;
216 the B73 group contains the other 25 founders: B73, B97, CML103, CML228, CML277,
217 CML322, CML333, CML52, CML69, HP301, Il14H, Ki11, Ki3, Ky21, M162W, M37W,
218 Mo18W, MS71, NC350, NC358, Oh43, Oh7B, P39, Tx303, and Tzi8. (B) Polymorphisms
219 upstream of *pebp8* correspond to a significant difference in DTA intercept (B97 group contains
220 B97, Il14H, MS71, Oh7B, P39, and Tx303; B73 group contains B73, CML103, CML228,
221 CML247, CML277, CML322, CML333, CML52, CML69, HP301, Ki11, Ki3, Ky21, M162W,
222 M37W, Mo18W, NC350, NC358, Oh43, and Tzi8).

223

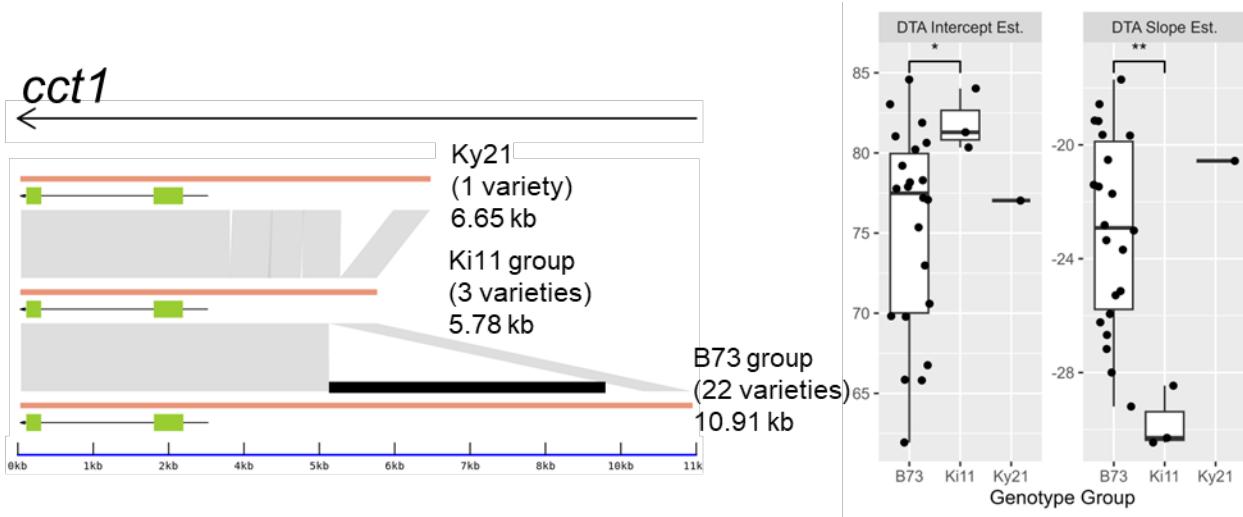


225 Fig. S12: BRIDGEcereal visualization of major haplotypes for DTA slope candidate genes and
 226 associated phenotype estimates among the NAM founders. (A) A small insertion is present
 227 upstream of *conz1* but is not significantly associated with a change in slope (B97 group contains
 228 B97, CML322, CML333, Ki11, and MS71; B73 group contains B73, CML103, CML228,
 229 CML247, CML277, CML52, CML69, HP301, Il14H, Ki3, Ky21, M162W, M37W, Mo18W,
 230 NC350, NC358, Oh43, Oh7B, P39, Tx303, and Tzi8). (B) An insertion within the second exon
 231 of *cry3* is significantly associated with a steeper DTA slope (B73 group contains B73, HP301,

232 Il14H, Oh7B, Oh43, and P39; B97 group contains B97, CML103, CML228, CML247, CML277,
233 CML322, CML333, CML52, CML69, Ki11, Ki3, Ky21, M162W, M37W, Mo18W, MS71,
234 NC350, NC358, Tx303, and Tzi8)

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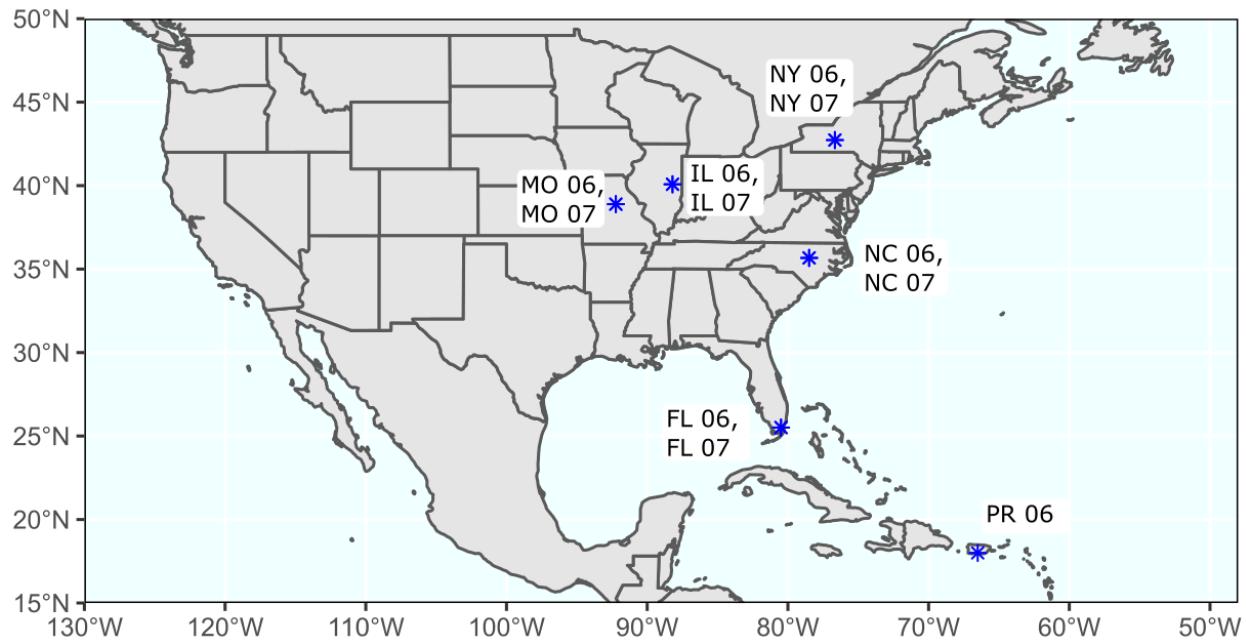


237

238 Fig. S13: BRIDGEcereal visualization of major haplotypes for *cct1*, the selected candidate gene
 239 identified for both DTA slope and intercept, and associated phenotype estimates among the
 240 NAM founders. The CACTA-like insertion identified upstream of *cct1* in CML228, CML277,
 241 and Ki11 (Ki11 group) is a known allele that reduces *cct1* expression and thereby reduces
 242 flowering time (DTA intercept). Here, this allele was also significantly associated with a steeper
 243 DTA slope. In addition, a potential weak allele was identified in Ky21 (single member of Ky21
 244 group). B73 group contains B73, B97, CML103, CML247, CML322, CML333, CML52,
 245 CML69, HP301, Il14H, Ki3, M162W, M37W, Mo18W, MS71, NC350, NC358, Oh43, Oh7B,
 246 P39, Tx303, and Tzi8.

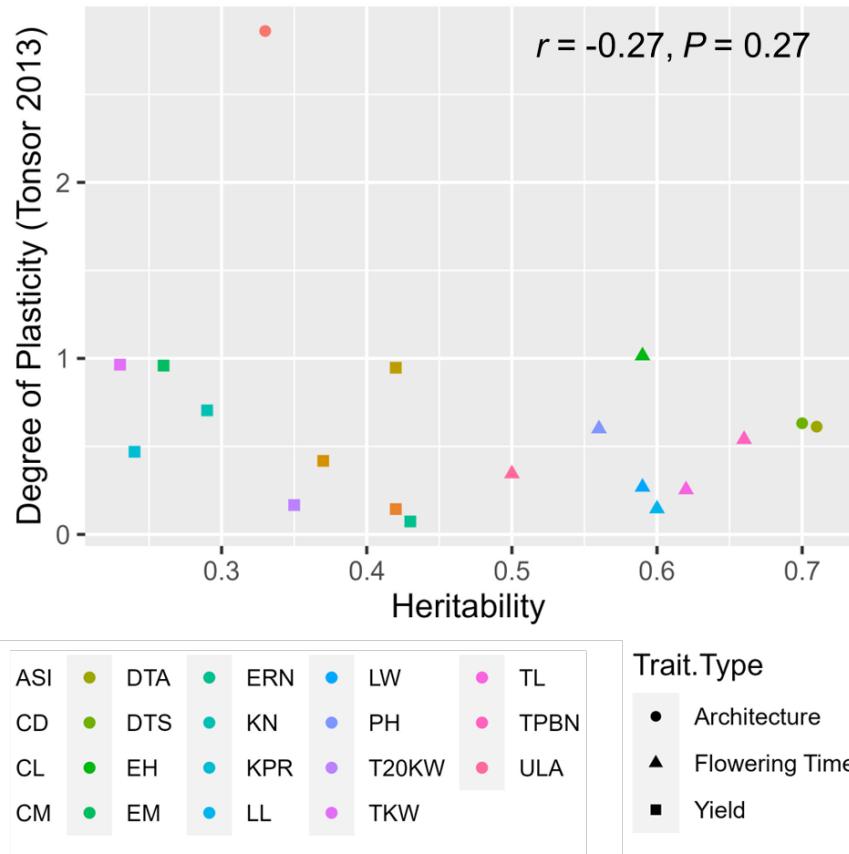
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249 Fig. S14: Study environments map. Data was collected at 6 locations (blue asterisks) over two
250 years. Environment names consist of the two-letter state abbreviation and the last two digits of
251 the year (e.g., MO06 is Missouri 2006).

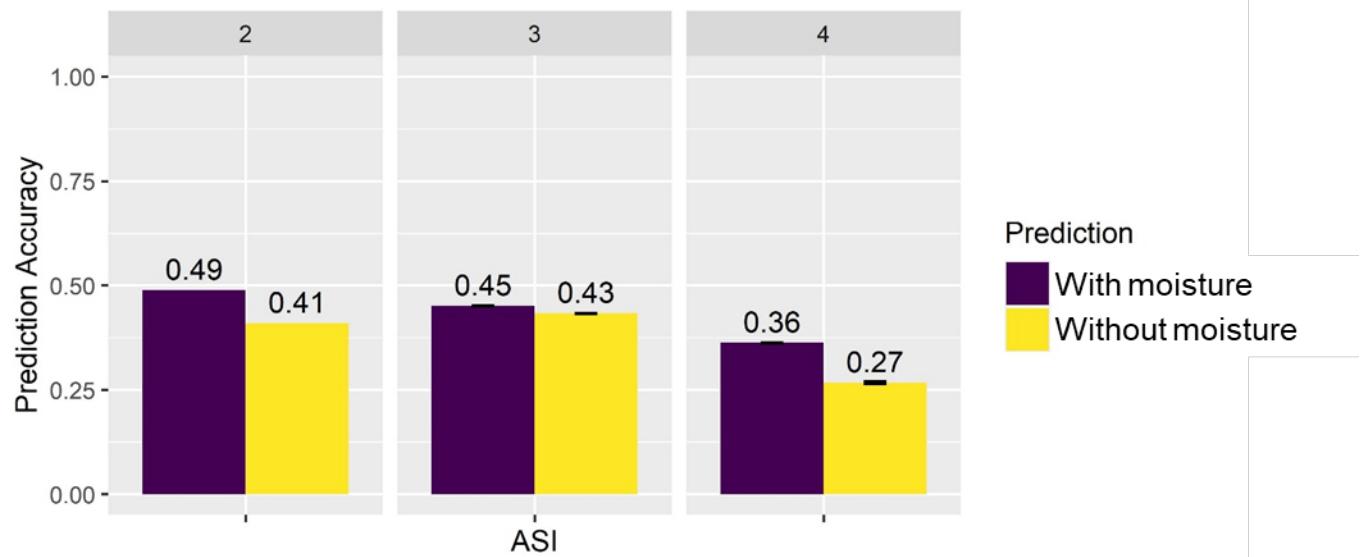
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253

254 Fig. S15: Heritability (h_p^2) and degree of plasticity (calculated per Tonsor et al. 2013) are not
 255 significantly correlated in the maize NAM.

256



257

258 Fig. S16: Prediction accuracy for Anthesis-Silking Interval (ASI) with and without moisture
 259 variables. When including variables involving moisture (precip, PET, and water balance) in the
 260 CERIS search space (purple), prediction accuracy improved significantly for ASI. Error bars
 261 show standard error of prediction accuracy based on 30 replicates with and 5 replicates without
 262 moisture variables.

263

Supplemental Tables

265 Table S1: List of traits. Abbreviations, full names, types, number of environments measured in (#
 266 Envs), measurement time, and search windows for all nineteen traits examined in this study.
 267 Measurement time indicates the time during the growing season at which this trait was measured,
 268 and search window indicates the range of days after planting that were searched by CERIS for
 269 this trait to ensure that no prediction was based on a window after the trait had been measured.

Trait Abbreviation	Trait Name	Trait Type	# Envs	Measurement Time	Search Windows
ASI	Anthesis-Silking Interval	Flowering Time	11	Flowering Time	1 – 46
CD	Cob Diameter	Yield	6	Harvest	1 – 106
CL	Cob Length	Yield	6	Harvest	1 – 106
CM	Cob Mass	Yield	6	Harvest	1 – 106
DTA	Days to Anthesis	Flowering Time	11	Flowering Time	1 – 46
DTS	Days to Silking	Flowering Time	11	Flowering Time	1 – 46
EH	Ear Height	Plant Architecture	11	Flowering Time	1 – 46
EM	Ear Mass	Yield	6	Harvest	1 – 106
ERN	Ear Row Number	Yield	6	Harvest	1 – 106
KN	Kernel Number	Yield	5	Harvest	1 – 106
KPR	Kernels per Row	Yield	6	Harvest	1 – 106
LL	Leaf Length	Plant Architecture	9	Flowering Time	1 – 46
LW	Leaf Width	Plant Architecture	9	Flowering Time	1 – 46
PH	Plant Height	Plant Architecture	11	Flowering Time	1 – 46
T20KW	Weight of 20 Kernels	Yield	5	Harvest	1 – 106
TKW	Total Kernel Weight	Yield	6	Harvest	1 – 106
TL	Tassel Length	Plant Architecture	8	Flowering Time	1 – 46
TPBN	Tassel Branch Number	Plant Architecture	8	Flowering Time	1 – 46
ULA	Upper Leaf Angle	Plant Architecture	9	Flowering Time	1 – 46

270 Table S2: CERIS-chosen environmental indices. Environmental indices identified by CERIS for
 271 each trait using all available data. For each trait, the environmental index consists of a window
 272 and environmental variable (env.variable). The window's start and end are presented as days
 273 after planting (DAP). The correlation between the chosen environmental index (EI) and the
 274 environmental mean (EM) is shown as $r_{EI,EM}$.

Trait Abbreviation	Start (DAP)	End (DAP)	Env. Variable	$r_{EI,EM}$
ASI	7	18	PRECIP	-0.8773
CD	6	38	PET	-0.9981
CL	72	81	PTD1	0.9930
CM	78	85	PTD1	0.9998
DTA	31	39	PTR	-0.9964
DTS	31	39	PTR	-0.9964
EH	26	41	PET	0.9201
EM	98	105	PTD1	0.9988
ERN	90	96	DTR	0.9822
KN	97	103	PTD1	0.9987
KPR	92	103	PTS	0.9879
LL	16	40	PRECIP	0.9645
LW	23	38	PRECIP	0.8233
PH	34	40	PET	0.8889
T20KW	40	73	GDD	0.9989
TKW	93	102	PET	0.9991
TL	32	41	PTD2	-0.8851
TPBN	30	43	PET	0.8772
ULA	7	38	PRECIP	0.7363

275
 276
 277

278 Table S3: Significant markers. Contains markers detected as significant by GWAS for each trait
279 (slope and intercept) using a SimpleM threshold ($\alpha = 0.05$). The first three columns contain
280 chromosome, base pair location, and name for each significant marker. Subsequent columns are
281 traits. A blank (NA) cell indicates that the marker was not detected for that trait, while numbers
282 indicate *P* values for significant markers.

283

284 Note: Table S3 is attached as “Supplemental_Table_S3.csv”

285

286 Table S4: Candidate genes. Contains candidate genes detected by GWAS for each trait (slope
287 and intercept) using a 20kb window around each significant marker. The first column contains
288 all gene names from Zm-B73-REFERENCE-NAM-5.0 which were significant for at least one
289 trait and the first row contains trait names. A “1” in a cell means that that gene was detected as a
290 candidate gene for that trait, and a “0” means that it was not.

291

292 Note: Table S4 is attached as “Supplemental_Table_S4.csv”

293

294 Table S5: Enriched GO terms. Cells contain g:SCS multiple testing correction adjusted *p* values;
295 cells with significant values are shaded gray and marked with an asterisk. Candidate genes
296 within 20kb of significant markers from slope and intercept GWAS were analyzed for GO term
297 enrichment. This analysis was conducted for the combined gene lists from all traits as well as
298 within trait groups (Flowering Time, Yield, and Plant Architecture).

GO Term Name	GO Term ID	All Traits		Flowering Time		Plant Architecture		Yield	
		Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope
Ubiquinol:oxygen oxidoreductase activity	GO:0102721	*0.00004	1.00000	1.00000	1.00000	*0.00016	1.00000	1.00000	1.00000
Alternative oxidase activity	GO:0009916	*0.00004	1.00000	1.00000	1.00000	*0.00016	1.00000	1.00000	1.00000
Oxidoreductase activity, acting on diphenols and related substances as donors, oxygen as acceptor	GO:0016682	*0.00203	1.00000	1.00000	1.00000	*0.00069	1.00000	1.00000	1.00000
Oxidoreductase activity, acting on diphenols and related substances as donors	GO:0016679	*0.02732	1.00000	1.00000	1.00000	*0.00665	1.00000	1.00000	1.00000
RNA-directed 5'-3' RNA polymerase activity	GO:0003968	1.00000	0.09460	1.00000	*0.03676	1.00000	1.00000	1.00000	1.00000
ATPase-coupled intramembrane lipid transporter activity	GO:0140326	1.00000	*0.04653	1.00000	0.23530	1.00000	1.00000	1.00000	1.00000
Alternative respiration	GO:0010230	*0.00089	1.00000	1.00000	1.00000	*0.00214	1.00000	1.00000	1.00000
Detoxification	GO:0098754	1.00000	0.47270	1.00000	*0.03830	1.00000	1.00000	1.00000	1.00000
Response to toxic substance	GO:0009636	1.00000	0.56234	1.00000	*0.04288	1.00000	1.00000	1.00000	1.00000
Cellular oxidant detoxification	GO:0098869	1.00000	0.45173	1.00000	*0.04711	1.00000	1.00000	1.00000	1.00000
Plant hormone signal transduction	KEGG:04075	*0.02339	1.00000	1.00000	1.00000	*0.02313	1.00000	1.00000	1.00000
Biosynthesis of secondary metabolites	KEGG:01110	1.00000	*0.02882	1.00000	0.23211	1.00000	1.00000	1.00000	1.00000

300 Table S6: Within-environment heritability. Heritability on an individual plot basis (h_p^2) for each
 301 trait in each environment. Blank cells indicate environments in which the specified trait was not
 302 measured.

	IL06	FL06	MO06	NC06	NY06	PR06	IL07	FL07	MO07	NC07	NY07
ASI	0.24	0.44	0.20	0.55	0.42	0.43	0.25	0.63	0.67	0.59	0.34
CD	0.39	0.53		0.64	0.60	0.59				0.77	
CL	0.45	0.40		0.62	0.56	0.53				0.73	
CM	0.66	0.46		0.64	0.69	0.58				0.77	
DTA	0.86	0.79	0.81	0.86	0.85	0.71	0.87	0.88	0.93	0.92	0.70
DTS	0.87	0.74	0.83	0.85	0.69	0.77	0.84	0.83	0.92	0.91	0.66
EH	0.76	0.68	0.60	0.79	0.66	0.67	0.75	0.73	0.77	0.77	0.77
EM	0.55	0.42		0.58	0.61	0.55				0.81	
ERN	0.64	0.60		0.69	0.57	0.58				0.77	
KN		0.54		0.58	0.69	0.59				0.84	
KPR	0.22	0.34		0.53	0.63	0.46				0.72	
LL	0.80	0.71	0.65	0.71	0.67	0.65	0.73			0.65	0.59
LW	0.65	0.66	0.59	0.68	0.54	0.69	0.71			0.75	0.62
PH	0.83	0.71	0.65	0.83	0.58	0.70	0.76	0.69	0.83	0.77	0.68
T20KW		0.39		0.61	0.61	0.65				0.76	
TKW	0.41	0.42		0.56	0.63	0.54				0.81	
TL	0.77	0.72	0.66	0.78	0.70	0.71	0.76				0.73
TPBN	0.73	0.62	0.71	0.72	0.63	0.75	0.70				0.67
ULA	0.60	0.62	0.39	0.68	0.62	0.45	0.63			0.78	0.58

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