

Reshaping of the maize transcriptome by domestication

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Through domestication, humans have substantially altered the morphology of *Zea mays* ssp. *parviglumis* (teosinte) into the currently recognizable maize. This system serves as a model for studying adaptation, genome evolution, and the genetics and evolution of complex traits. To examine how domestication has reshaped the transcriptome of maize seedlings, we used expression profiling of 18,242 genes for 38 diverse maize genotypes and 24 teosinte genotypes. We detected evidence for more than 600 genes having significantly different expression levels in maize compared with teosinte. Moreover, more than 1,100 genes showed significantly altered coexpression profiles, reflective of substantial rewiring of the transcriptome since domestication. The genes with altered expression show a significant enrichment for genes previously identified through population genetic analyses as likely targets of selection during maize domestication and improvement; 46 genes previously identified as putative targets of selection also exhibit altered expression levels and coexpression relationships. We also identified 45 genes with altered, primarily higher, expression in inbred relative to outcrossed teosinte. These genes are enriched for functions related to biotic stress and may reflect responses to the effects of inbreeding. This study not only documents alterations in the maize transcriptome following domestication, identifying several genes that may have contributed to the evolution of maize, but highlights the complementary information that can be gained by combining gene expression with population genetic analyses.

The domestication of maize from its wild progenitor is a model system for investigating domestication, genome evolution, and response to selection (1–4). Cytogenetic and molecular analyses of maize domestication have identified *Zea mays* ssp. *parviglumis* (hereafter teosinte) as the direct wild progenitor of maize and indicate these lineages diverged ~9,000 generations ago (5, 6). Despite their recent divergence, maize exhibits substantial phenotypic differences from its wild progenitor, reflecting rapid and pronounced evolutionary change (2, 5). Identification of genetic changes underlying these phenotypic differences will give insight into the genetic architecture of complex traits (3, 7), characterize response to selection (8), and provide resources for maize improvement (3).

Both quantitative trait locus (QTL) mapping and molecular population genetic scans have identified numerous genomic regions that underlie maize domestication (3, 9–14). Molecular characterization of QTL has identified genes that appear to be responsible for several of the morphological or phenological differences between maize and teosinte, including *tb1* (15), *tga1* (16, 17), *zfl2* (18), *ba1* (19), and *ra1* (20). A number of genes with putative regulatory function have been identified as potential domestication genes (3, 13), and a recent genome-wide analysis of maize and teosinte identified numerous selected regions devoid of annotated genes (14). These data are consistent with suggestions that regulation of gene expression has played an important role in the evolution of maize (3, 21–23). The importance of regulatory, as opposed to structural gene, changes is also

consistent with the broader hypothesis that regulatory differences are fundamental to the evolution of morphological and developmental diversity (24).

To investigate the evolution of gene expression that accompanied maize domestication, we examined the transcriptome of 38 diverse maize inbred lines and 24 teosinte accessions. Our specific objectives were twofold. First, we tested for significant between-taxa differences in expression, which may occur as a result of directional selection. Second, we used coexpression network analyses to test whether domestication has rewired the transcriptional network, causing changes in the covariance of gene expression. We find evidence for significant changes in both gene expression levels and coexpression relationships following domestication and identify a subset of genes with altered expression patterns that were also likely targets of selection during domestication.

Results

Using a NimbleGen (Roche NimbleGen) expression array representing 32,540 genes [the filtered gene set annotation 4a.53 of the maize reference genome from Schnable et al. (25)], we collected expression profile data from 8-d-old tissue of 62 genotypes: 38 diverse maize inbred lines, 7 teosinte inbred lines, and 17 teosinte individuals sampled from three wild-collected, outcrossing populations (Table S1). Eight-day-old plants, which have cotyledons and one or two leaves (Fig. S1A), were chosen for expression profiling to minimize expression differences attributable to developmental disparity between genotypes and taxa. Of the 32,540 genes represented on the array, we first identified 19,792 genes (73,104 oligonucleotides) that showed evidence for expression based on hybridization signal above levels of random nonmaize control sequences. We eliminated an additional 26,937 probes that comparative genomic hybridization (CGH) data (26) showed to have high cross-genotype variation in hybridization signal for genomic DNA, presumably attributable to nucleotide divergence or structural variation. Our final dataset consisted of 46,167 CGH-filtered probes representing 18,242 expressed genes (1–4 probes per gene) that were Robust Multichip Average (RMA) normalized (Dataset S1) and used for subsequent analyses.

Genome-wide, the coefficient of variation of expression among lines was nearly identical in maize and teosinte (Fig. 1A), indicating artificial selection did not cause a transcriptome-wide

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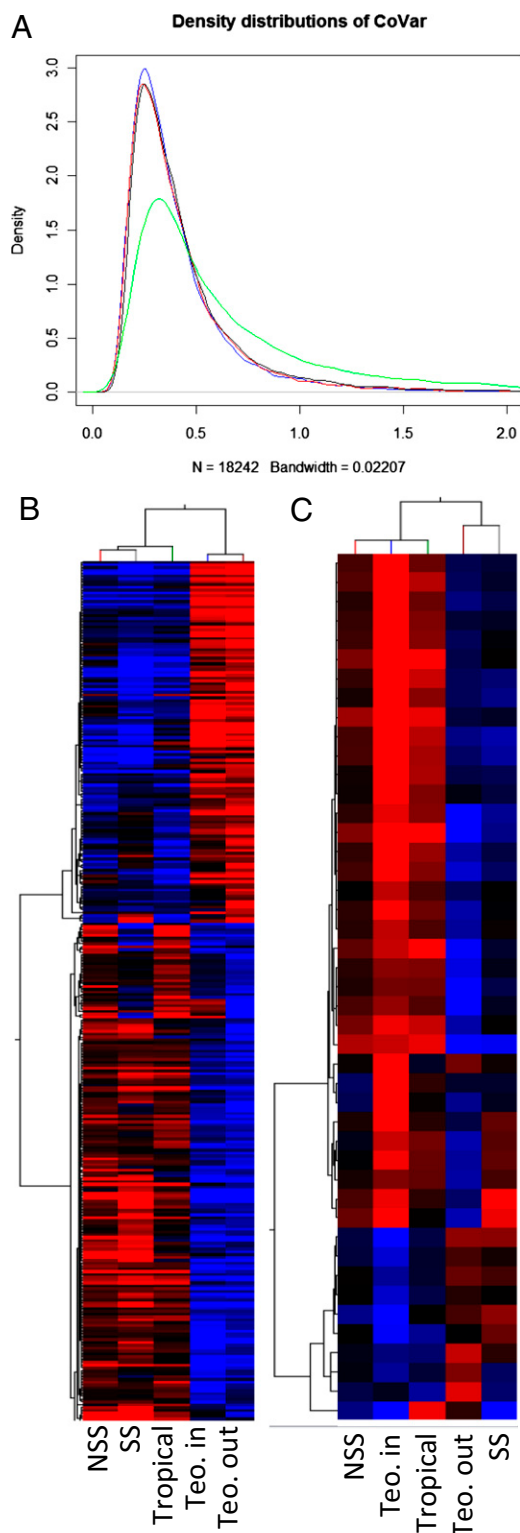


Fig. 1. Variance in expression. (A) Density plots for the coefficient of variance (CoVar) for gene expression levels in all genotypes (black), maize genotypes (red), and teosinte genotypes (blue), as well as for developmental stages (green). More genes exhibit a higher CoVar across developmental stages than across diverse genotypes. (B) Relative gene expression levels for the 612 genes with significant expression differences between maize and teosinte, and used for hierarchical clustering. Genotypes were each assigned to one of five subpopulations (specified in [Table S1](#)). (C) Similar clustering is shown for the 45 genes that are differentially expressed between inbred and outcrossed teosinte. NS5 (nonstiff stalk): SS (stiff stalk).

reduction in the variation of expression, nor has subsequent maize improvement resulted in vastly different expression levels among inbred lines. Expression varied more among tissues and developmental stages [data from Sekhon et al. (27)] than among different genotypes (Fig. 1A and Fig. S1 B and C). Among-line variance of expression differed significantly with respect to various factors, including gene conservation, genomic locations, and gene family size (Fig. S1 D–F).

We identified 612 genes with significantly different [posterior probability ($P_{\text{posterior}}$) of differential expression (DE) > 0.999] levels of expression in maize compared with teosinte (Table 1), with nearly half of these genes ($n = 288$) showing at least twofold difference in expression, with a slight bias (58.3%) toward higher expression in maize. Hierarchical clustering of the relative expression of these genes that show DE demonstrates that for some genes, there are groups of maize genotypes with expression levels similar to those in teosinte (Fig. 1B).

Teosinte plants normally exhibit low levels of self-fertilization (28), but modern maize breeding programs are now based on creating hybrids between inbred lines generated through controlled self-pollinations. All the maize lines included in our analyses are inbred lines. Seven of our teosinte genotypes (designated as TILs) are genetic stocks produced by multiple generations of self-fertilization. The remaining 17 teosinte samples are individuals from three wild-collected, outcrossing populations (Table S1). To investigate the effects of inbreeding on expression patterns, we compared expression between inbred and outbred teosinte. We identified 45 genes with significantly altered expression in outcrossed relative to inbred teosinte plants ($P_{\text{posterior}} > 0.999$; Table 1), with the majority of these expressed at higher levels in inbred plants (Fig. 1C). Seven of these 45 genes also showed significant differences in expression levels between maize and teosinte (Dataset S1). Among these 45 DE genes, there is significant enrichment for chitin metabolic processes and defense response genes, all of which were more highly expressed in inbred plants (Table S2). Many of these DE genes also are members of the same teosinte coexpression subnetwork that is enriched for genes annotated with biological functions in response to biotic stimulus (Table S2).

Rewiring of Transcription in Maize Relative to Teosinte. DE analyses identify individual genes with significantly different expression but may not identify changes in regulatory relationships among pairs or groups of genes. Analysis of coexpression across a set of genotypes can, however, be used to identify genes whose coregulation was altered during domestication, even if those genes' average expression was not significantly changed. To investigate coexpression relationships on a global scale, we compared the topologies of coexpression networks that were separately constructed for maize and teosinte. To quantify the coexpression within each taxon, we generated a coexpression matrix by calculating the among-genotype correlations for every pair of genes within each taxon, and these correlations are the edges of the network. We then examined the correlation between edges in the two networks;

Table 1. DE genes

Gene list	Gene no.	% 2FC	% up-regulated in maize	No. Dom or Imp candidates
Maize vs. teosinte DE	612	47	58.30	90
Teosinte inbred vs. outcrossed DE	45	95	NA	4
AEC	1,115	16	57.1	135
AEC and DE	276	51	63.4	46

Dom, domestication; 2FC, 2 fold-change; Imp, improvement; NA, Not applicable.

a correlation of 1 would indicate that the patterns of coexpression in maize were identical to coexpression in teosinte. The empirical correlation between edges in the maize and teosinte networks was 0.30, which was lower than the correlation observed in all but 14 of 1,000 pairs of matrices derived from random permutations of the genotypes (Fig. 2A). The rewiring of expression networks since maize-teosinte divergence is also evident from pairwise gene expression correlations, which reveal far fewer conserved gene pairs than expected based on resampled coexpression networks (Fig. 2B and C).

To identify genes contributing the largest differences between the maize and teosinte coexpression networks, we computed an expression conservation (EC) score for each gene, a measure of the degree of similarity between a candidate gene's neighbors in the maize expression network and the same gene's neighbors in the teosinte network (*Materials and Methods*). Consistent with the correlation analyses, we observed a transcriptome-wide shift in EC score toward lower conservation, relative to the null expectation (Fig. S24). To characterize the genes showing the strongest altered expression conservation (AEC) between maize and teosinte (hereafter referred to as AEC genes), we identified a set of 1,115 genes with observed EC scores >3 SDs below the

expected EC score derived from random permutations of the genotypes.

Characterization of Genes with Altered Expression in Maize and Teosinte. The above analyses identified 612 genes with DE levels in maize and teosinte and 1,115 genes with AEC in maize and teosinte. Of these, 276 showed both DE and AEC, significantly more than expected by chance ($P < 0.05$). However, DE and AEC approaches identify partially distinct aspects of maize-teosinte expression changes (Fig. S2 B and C). A gene ontology (GO) analysis of genes that have reduced expression in maize relative to teosinte finds evidence for significant overrepresentation of genes related to amino acid salvage, cellular respiration, and sulfur amino acids biosynthetic processes (Fig. S3A). The genes with reduced expression in maize are also overrepresented by genes that are either not located in syntenic regions in sorghum or that are maize- or grass-specific (Fig. S3 B and C). However, the majority of DE genes are syntenic with rice and sorghum.

It is possible that some of the altered expression observed in maize relative to teosinte might represent differences in development or anatomy of the two taxa. To test for this possibility, we compared the DE and AEC genes with developmental coexpression networks derived from 60 different tissues/stages of B73 (27). We did not find evidence that the DE or AEC genes were enriched in specific developmental coexpression clusters, which suggests that neither the DE nor AEC genes are the result of differences in development or morphology of maize and teosinte.

Altered Gene Expression Within Targets of Selection. Transcriptome profiling can identify genes that are either responsible for differences between species or are downstream of causative changes. To identify expression changes that are potentially responsible for differences selected during domestication, we compared DE and AEC genes with a list of genes located in genomic regions putatively selected during domestication and/or improvement (14) (Fig. 3A). Genes that show both DE and AEC are significantly overrepresented among genes found in these candidate regions ($P < 0.05$; Table 2). These genomic regions are also enriched for DE-only genes but show no significant overrepresentation of AEC-only genes (Table 2). This may provide evidence that AEC-only genes are reflecting downstream rewiring of the transcriptome but are likely not the causal sequence variants on which selection occurred during domestication.

We focused our analyses on genes in selected regions that show both DE and AEC (46 genes in Table S3) or show DE only (44 genes in Table S4), because these lists were significantly enriched for putative targets of selection. Analysis of domains present within these genes and annotation of the closest match in *Arabidopsis* suggest that 13 of the 90 genes may function as transcription or chromatin factors. The majority of the 90 genes show higher expression in maize (35 of 46 for the DE and AEC genes, 27 of 44 for DE-only genes) and tend to have slightly higher connectivity in the teosinte coexpression network relative to maize (Table 2). Many of the genes show substantially altered levels of connectivity in maize and teosinte (Tables S3 and S4), but this difference in connectivity does not appear to be related to the directionality of change in expression. There also is not a clear or consistent pattern in the nature of the connectivity differences shown by these genes. Coexpression subnetworks were analyzed for several of these genes to understand better how domestication affected their coregulatory relationships (Fig. 3B and Fig. S44). These example teosinte coexpression networks include several small and moderate-sized networks. The gene used as the query for the networks (shown in red in Fig. 3B and Fig. S44) is highly connected in teosinte. However, many of the connections for this gene are lost following domestication. Examples are also found in which parts of the teosinte coexpression network are maintained in the maize network.

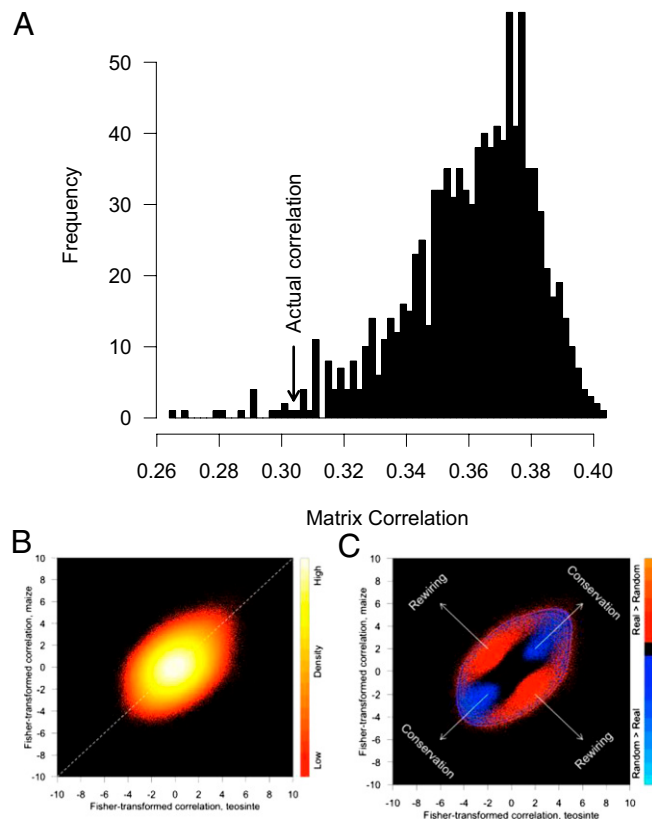


Fig. 2. Rewiring of transcriptional networks in maize and teosinte. (A) Pearson correlation coefficient was determined for the full matrix correlation of maize and teosinte coexpression networks (black arrow). Only 1.4% or 1,000 pairs of networks derived from randomly permuting the genotypes exhibit lower correlations than the maize and teosinte networks. (B) Scatterplot shows the correlation between all gene pairs in maize (x axis) relative to the correlation for the same gene pair in teosinte (y axis). The relative density of data points in B was compared with the average for 1,000 bootstrap coexpression networks in C. Blue regions indicate fewer observed correlations relative to the bootstrap networks, whereas red coloration indicates an excess of actual observations, providing evidence for an enrichment of gene pairs with varying correlations in maize and teosinte.

sweeps. Many of the genomic regions identified by Hufford et al. (14) contain multiple genes, and these investigators identified the most likely target of selection within these regions by choosing the gene nearest the point in the region with the highest likelihood for selection during domestication or improvement. Many of the DE genes within selected regions (28 of 90 genes) represent the gene identified as the candidate target of selection based on population genetic metrics (Fig. 3C, Table 2, and Tables S3 and S4). The other 62 examples of differentially expressed genes in these regions represent examples in which the differentially expressed gene was in the selected region but was not the gene with the highest likelihood of selection (example in Fig. 3D). Although these genes are not located nearest the selection likelihood peak, the DE observed makes them compelling candidates nonetheless.

An examination of the expression levels of DE genes in each of the maize and teosinte genotypes reveals that although the average maize and teosinte expression levels are quite different, there is frequent overlap in the range of expression levels in maize and teosinte, such that some teosinte genotypes have expression levels similar to those found in maize or vice versa. This observation could reflect selection occurring on standing variation in teosinte, resulting in an increase in allele frequency during domestication. Conversely, the finding that some maize genotypes have expression levels more similar to teosinte could reflect examples in which the selective sweep has not been complete and some maize genotypes still retain an alternative allele. To investigate these possibilities, we compared patterns of expression of several DE genes with genetic distances calculated from the SNP data of Hufford et al. (14) (Fig. 3E and Fig. S5 A and B). The neighbor-joining trees generated using these SNP data show that the majority of maize genotypes have a very similar allele. However, there are examples in which some maize individuals group with teosinte or in which some teosinte genotypes are most similar to maize. In general, the expression level is highly correlated with the allele that is present.

Discussion

Regulatory changes have been proposed to play a major role in phenotypic evolution and some of the best-characterized morphological changes that have accompanied domestication appear to have resulted from adaptive changes at transcription factors (3, 7, 8, 24). However, the extent to which domestication, or species divergence in general, has altered the transcriptome is not well understood. By profiling the transcriptomes of 38 maize and 24 teosinte individuals, we characterized the diversity of transcriptional variation within each of these taxa, as well as the divergence of expression that has occurred during the past ~9,000 y since domestication. The overall coexpression networks in maize and teosinte show evidence for significant rewiring. Subsequent per gene analyses identified many examples of both DE and AEC. The analysis of coexpression networks that have substantially changed in maize relative to teosinte may assist in further understanding the molecular basis of phenotypic adaptation. In addition, the comparison of transcriptomes of wild and domesticated derivatives can begin to describe how selection on quantitative traits has affected gene expression networks.

In a search for targets of maize domestication and improvement, Hufford et al. (14) identified 484 and 695 chromosomal regions with signals of selection during domestication and improvement, respectively. A large proportion of these selected regions (58% of domestication regions and 48% of improvement regions) include multiple genes. These investigators subsequently identified the most likely target of selection within these regions by identifying the gene nearest the point in the region with the highest likelihood of selection during domestication or improvement. Analysis of maize and teosinte transcriptomes can provide a complementary approach to characterize genes within selected

intervals further and to identify likely candidate targets. In some cases (28 of 90 cases), the two approaches identified the same gene, although for the remaining 62 windows of selection, the DE gene is not the gene nearest the region of the window with the greatest statistical support for being the target of selection. These windows may be examples of the limits of using population-genetic inferences alone for identifying targets of selection. Alternatively, the target of selection identified through population-genetic analyses may be correct and the altered expression may reflect a *cis*-regulatory variant that has “hitchhiked” along with the selected allele. Hufford et al. (14) identified 299 chromosomal regions that show evidence for selection during domestication or improvement but contain no genes from the filtered gene set. The nearest genes from the filtered gene set were compared with the 612 DE genes, and we identified five cases of DE for the genes located near selected regions (Fig. S5D). These may be examples for selection acting directly on regulatory sequences.

Although we do not have information on the functional consequences of the expression changes we detected, several DE genes are among the classic and well-studied maize genes [Schnable and Freeling (29)]. These include *ae1* (amylose extender1), *an1* (anther ear1), *adh2* (alcohol dehydrogenase2), *chn3* (chitinase3), *du1* (dull endosperm1), *fht1* (flavonone 3-hydroxylase 1), *gln2* (glutamine synthetase2), *lpa1* (low phytic acid1), and *zmm2* (*Z. mays* MADS2). Two of these genes, *adh2* and *zmm2*, also show evidence of selection during maize domestication or improvement in the study by Hufford et al. (14). Interestingly, *ae1* was previously identified as a target of selection during domestication [Whitt et al. (30)]. This gene is nearly twofold more highly expressed in teosinte seedlings relative to maize seedlings. The potential functional significance of additional DE genes also can be inferred based on orthology. For example, GRMZM2G448355, a domestication candidate [Hufford et al. (14)], has sequence similarity to rice *OsMADS56*, which is implicated in control of flowering time in rice and is located within a flowering-time QTL in maize (31).

An important limitation of the expression data we assayed is that they were collected from 8-d-old seedlings. We chose this developmental stage because all individuals, regardless of the taxon, are morphologically similar at this stage; therefore, differences we detect are likely attributable to divergence between taxa and not to comparing taxa that are at different developmental stages. An attempt to document differences in expression in visibly altered structures, such as flowers or seeds, would identify numerous expression differences that correspond to tissue differences and not necessarily expression per se. Although assaying 8-d-old seedlings allowed the isolation of comparable samples, it limited our ability to identify the targets of selection that are specifically expressed in other tissues that have been subjected to strong selective pressures. For example, only 2 (*ae1* and *su1*) of the 13 “known” targets of selection during domestication [Hufford et al. (14)] were expressed in seedling tissue; the remaining 11 genes, including *tb1* and *tg1*, are expressed specifically in other tissues (15, 16). Nevertheless, we identified differentially expressed genes in young seedling tissue that include a disproportionate amount of domestication or improvement candidates, as well as genes previously shown to play important functional roles in maize.

Despite the challenges of comparing the transcriptomes of domesticated plants and their wild ancestors, this approach can provide a detailed view of the impacts of selection on gene expression patterns. Even in young seedling tissue, before visible differences between maize and teosinte are apparent, we find evidence for significant rewiring of expression networks and find numerous differentially expressed genes. Previous studies have found that domestication targets are enriched for genes with regulatory functions (3, 21–23). This study has provided further evidence that domestication has frequently selected for regulatory variants and can also provide the basis to characterize the downstream targets of these genes.

Materials and Methods

Microarray Hybridization and Data Processing. Diverse maize inbred lines ($n = 38$), inbred teosinte lines ($n = 7$), and wild teosinte individuals ($n = 17$) were grown, and seedling leaf tissue was harvested 8 d after planting as previously described (26). Purified RNAs were labeled (details provided in *SI Materials and Methods*) and hybridized to a custom long-oligonucleotide microarray (GPL10846) designed by NimbleGen (Roche NimbleGen). The data were filtered to omit probes without substantial expression signal and probes with CGH variation (*SI Materials and Methods*). The raw data from the remaining 46,167 probes were renormalized using RMA (32) to provide the estimates of gene expression for 18,242 genes. Differentially expressed genes were identified using Cyber T (33) utilizing a conservative experiment-wide $P_{\text{posterior}} > 0.999$. The gene expression data generated for this study are available in the Gene Expression Omnibus database (accession no. GSE30036).

Coexpression Network Analysis. Coexpression networks were computed separately for the 38 maize expression profiles and 24 teosinte profiles by calculating Pearson correlation coefficients between all pairs of genes. Each set of correlations was then transformed using the Fisher transformation as recommended by Huttenhower et al. (34) and standard-normalized to allow for comparisons between the two networks. An EC score was calculated as the Pearson correlation coefficient between gene profiles in two coexpression networks as described by Dutilh et al. (35). The significance of differences between the maize and teosinte coexpression networks was assessed through bootstrapping, where genotypes were selected at random without replacement from the full-expression dataset, forming two groups with 38 and 24 genotypes each to match the number of genotypes in maize and teosinte subsets, respectively. Each obtained pair of subsets was used to build a coexpression network, and comparisons of the two networks were repeated on each of these bootstrapped networks. This process was repeated 1,000 times to generate null distributions for cross-network

correlation, the joint edge weight distribution, and an EC score distribution for each gene. Rewired genes were selected by computing a z score using the gene-specific null distribution and applying a cutoff of $z < -3.0$. Further details of how the coexpression networks were constructed and compared are included in *SI Materials and Methods*.

Analysis of Overlap Between Expression-Derived and Sequence-Derived Selection Target Lists. Hufford et al. (14) performed sequence-based analysis to identify 3,040 genes in regions targeted during domestication and improvement, of which 1,761 genes were present in our dataset. We measured the enrichment in the sequence-derived selection targets using the hypergeometrical distribution for each of the following sets of genes: DE-only genes, AEC-only genes, and genes that are both DE and AEC.

Expression levels in DE and DE/AEC genes were compared with genetic distance between genotypes using SNP data from Hufford et al. (14). Neighbor-joining trees were constructed based on simple parsimony substitution models as implemented in the program TASSEL (version 3.0) (36).

GO Analyses. The GOSlim annotation of gene lists was assessed using BiNGO (37), a Cytoscape (38) plug-in that maps overrepresented functional themes present in a given gene set onto the GO hierarchy. P values for enrichment of GOSlim terms were calculated using a hypergeometrical distribution statistical testing method with false discovery rate correction.

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Supporting Information

Swanson-Wagner et al. 10.1073/pnas.1201961109

SI Materials and Methods

DNA Labeling and Microarray Hybridization. A custom long-oligonucleotide microarray (GPL10846) was designed by NimbleGen (Roche NimbleGen) using the 32,540 filtered maize genes predicted from the B73 reference genome (1) as described by Swanson-Wagner et al. (2). RNAs were isolated using the commercial TRIzol (catalog no. 15596026; Invitrogen) from above-ground tissue harvested 8 d after germination. RNAs were purified by lithium chloride treatment, followed by 3 M sodium acetate (0.1 vol) and 95% (vol/vol) ethanol (2.5 vol) precipitation. Purified RNAs (10 μ g per sample) were reverse-transcribed and labeled according to the array manufacturer protocol (NimbleGen Arrays User's Guide: Gene Expression Analysis v3.2; Roche NimbleGen). Per sample, ~20 μ g of Cy3- or Cy5-labeled RNAs was hybridized for 16–20 h at 42 °C using the NimbleGen Hybridization System (Roche NimbleGen). After hybridization, slides were washed (NimbleGen Wash Buffer Kit; Roche NimbleGen) and dried for 2 min by centrifugation. Slides were immediately scanned using the GenePix 4000B Scanner (Molecular Devices) according to the array manufacturer's protocol.

Data Processing. Array images and data were processed using NimbleScan (Roche NimbleGen) software. Briefly, images from each slide were separated into 12 subarrays and aligned to a grid to extract signal intensity for each feature on the array. Experimental integrity was verified by evaluation of the signal intensities of the sample tracking control features for each subarray. Furthermore, metrics reports were produced for each array to report the signal uniformity across the array and the intensity of known empty features, random probes, and experimental probes. A total of 78 samples provided high-quality data and were used for subsequent analyses. NimbleScan was used to generate robust multichip average (RMA) normalized (3) gene expression values from the spatially corrected probe signal intensities on a per-probe and per-gene basis. Normalized gene expression values across multiple replications (technical or biological) of the same genotype were averaged when possible. Comparisons of the distributions of signal intensity for the random sequence controls compared with the experimental gene probes on each array were used to determine a reasonable signal threshold for positive expression across all slides. There are 19,792 genes (represented by 73,104 probes) that exhibit detectable signal (significantly higher than noise measurements) in at least three genotypes. The comparative gene hybridization (CGH) ratio (genomic DNA signal relative to B73 genomic DNA) was assessed for the 73,104 probes from expressed genes in hybridizations of over 40 diverse maize and teosinte genotype (2). Probes ($n = 26,937$) that exhibit low CGH values in at least three genotypes were eliminated, resulting in a set of 46,167 probes that measure expression in 18,242 genes (1–4 probes per gene). The raw data from these probes were renormalized utilizing RMA to provide the estimates of gene expression used for this study. Differentially expressed genes were identified using Cyber T (4) utilizing a conservative experiment-wide posterior probability of differential expression >0.999. The gene expression data generated for this study are available in the Gene Expression Omnibus database (accession no. GSE30036).

Construction of Coexpression Networks. For the generation of coexpression networks, we arranged the expression data into a matrix E that had 62 genotype columns and 18,242 gene expression profile rows. Each element E_{ij} denoted the expression

level of gene i in genotype j . We split the dataset by taxon into E_M and E_T subsets of maize (18,242 $\times$ 38) and teosinte (18,242 $\times$ 24) genotypes, respectively. To build coexpression networks represented by matrices R_M and R_T , we calculated the Pearson correlation coefficient between each pair of gene expression profiles within a single subset:

$$R_{ij}^M = PCC(E_i^M, E_j^M)$$

$$R_{ij}^T = PCC(E_i^T, E_j^T)$$

for $i, j = 1, \dots, 18,242$ and $i \neq j$. Thus, R_M and R_T were square matrices with the same dimensions, (18,242 $\times$ 18,242). Each value in those matrices represented an edge weight in the coexpression network and measured similarity between expression profiles of two genes. Even though the matrices had identical dimensions, the distribution of values in each matrix might be different because of the unequal sample size. Hence, a value from one matrix could not be compared directly with a value in the other. To enable direct comparison between R_M and R_T , we applied Fisher z transformation to both matrices as recommended by Huttenhower et al. (5). For each element r in R_M or R_T , the Fisher transformation is defined as

$$z = \frac{1}{2} \ln \frac{1+r}{1-r},$$

which guarantees that the distribution of z values is approximately normal. We normalized the distribution by subtracting the mean and dividing by the SD to obtain a standard normal distribution $N(0, 1)$. Thus, each element in R_M and R_T represented how many SDs the corresponding pairwise similarity score was from the mean, making cross-network comparison possible. The coexpression networks were created, processed, and analyzed using the SlepniR C++ library (6).

Comparison of Coexpression Networks. An expression conservation (EC) score was calculated as the Pearson correlation coefficient between gene profiles in two coexpression networks as described by Dutilh et al. (7):

$$EC = PCC(R_i^M, R_i^T),$$

where R_i^M and R_i^T were i -th rows in the matrices that represented maize and teosinte coexpression networks, respectively. For each round of bootstrapping ($n = 1,000$), genotypes were selected at random without replacement from the full expression dataset forming two groups with 38 and 24 genotypes each to match the number of genotypes in maize and teosinte subsets, respectively. Each obtained pair of subsets was then used to build a coexpression network. Because each group had a random mix of maize and teosinte accessions, any variations between coexpression networks would exist mainly as a result of random variation and the difference in group size. The same analysis was performed on each corresponding pair of the bootstrapped coexpression networks, including the calculation of matrix correlation, edge weight distribution, and EC score distribution. Null expectations for EC score and joint edge weight distribution were calculated as averages across all bootstraps. We selected rewired genes using a z score calculated for each EC value as follows:

$$z = \frac{EC - \mu}{\sigma},$$

where μ and σ were the mean and SD of the gene's EC scores obtained from the bootstrapping analysis. The z score of rewired genes was required to be below -3.0 .

Gene Annotations and Gene Ontology Analyses. The maize-specific genes and gene families were identified based on homolog clustering with annotated genes of rice, sorghum, and *Arabidopsis* using the method of Vilella et al. (8) as previously described (2). Pa-

ralogous clusters were defined as two or more genes belonging to the same gene family that were separated on a chromosome by no more than two nonparalogous intervening genes. Syntenic mapping of maize genes to rice and sorghum was previously described (2). The gene ontology (GO) GOslim annotation of genes that were affected by structural variation was assessed using BiNGO (9), a Cytoscape (10) plug-in that maps overrepresented functional themes present in a given gene set onto the GO hierarchy. P values for enrichment of GOslim terms were calculated using a hypergeometrical distribution statistical testing method with false discovery rate correction.

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10. Shannon P, et al. (2003) Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res* 13:2498–2504.

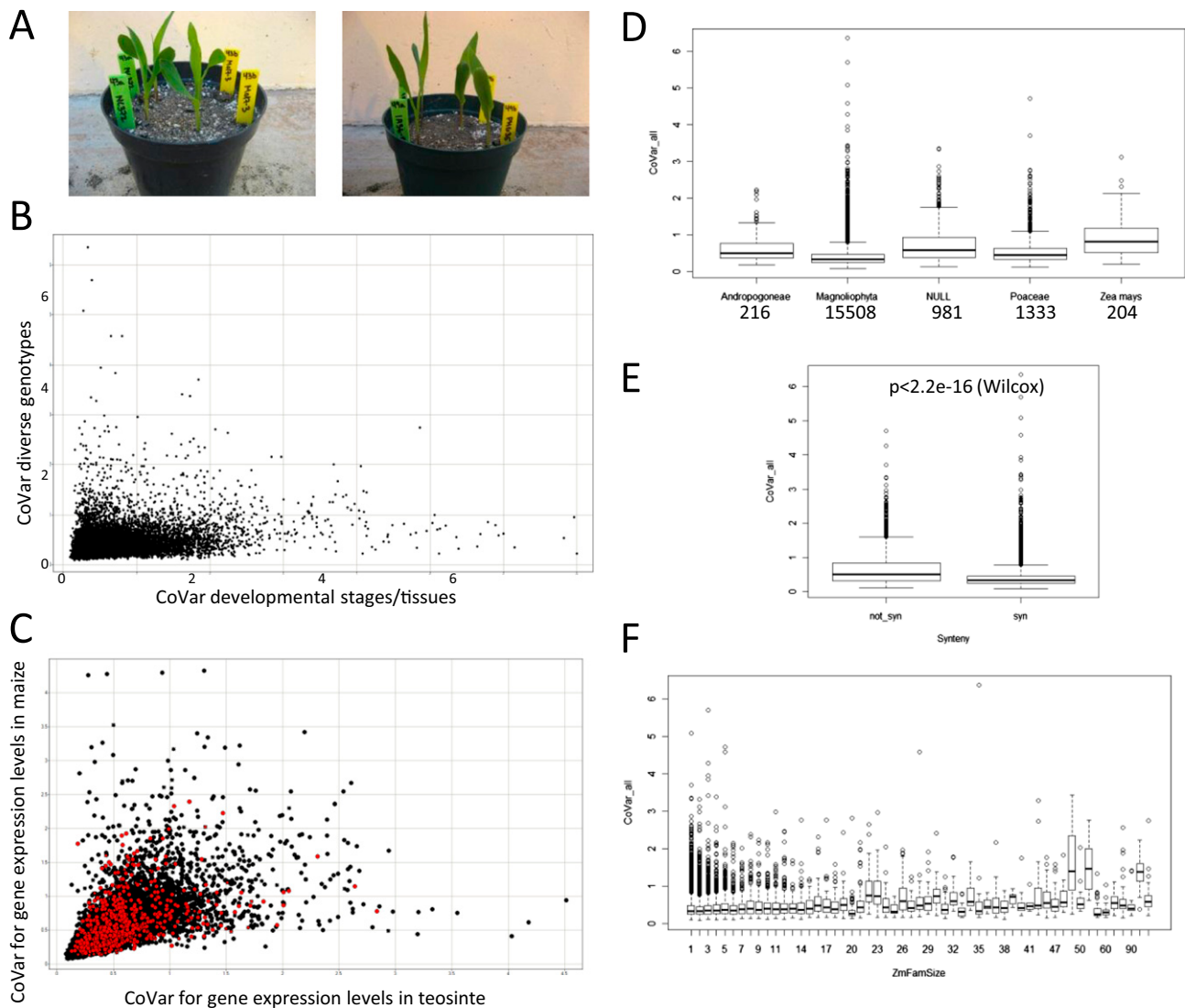
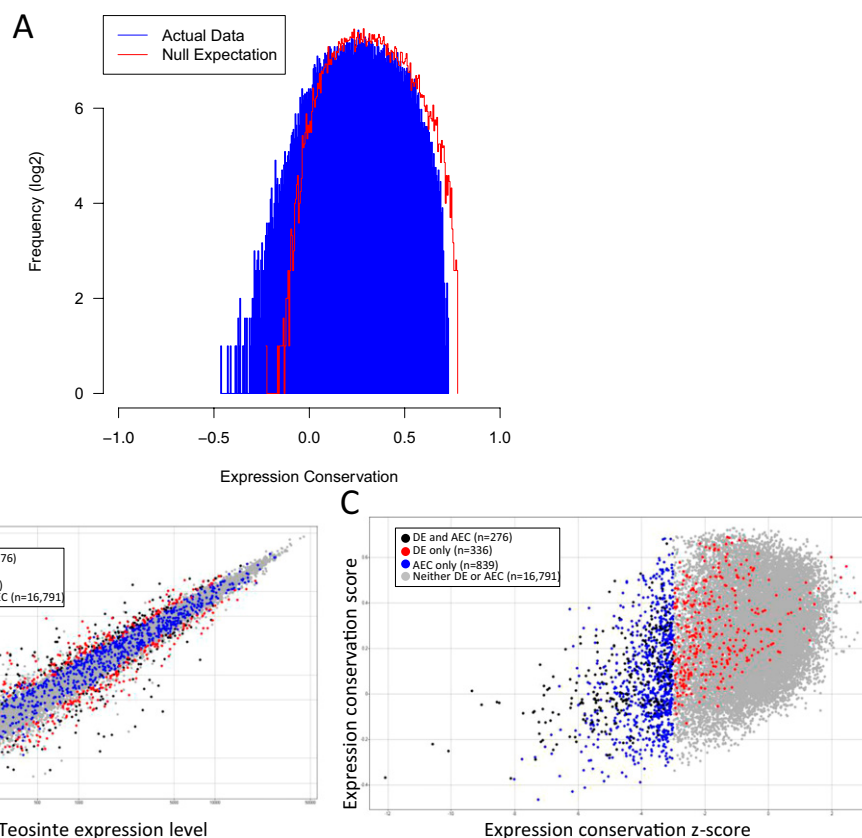
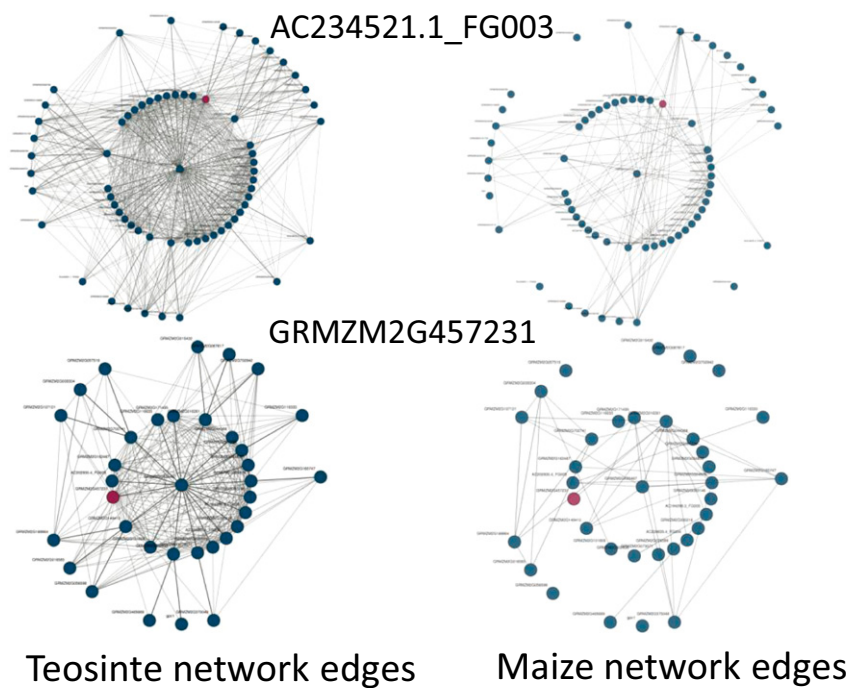


Fig. S1. (A) Images of sampled plants. Several images of 8-d-old plants immediately before harvesting for RNA isolation. (B) Coefficient of variance (CoVar) for gene expression levels was compared for 13,789 genes in a survey of different genotypes (y axis) and different developmental stages (x axis). (C) CoVar for gene expression levels in all maize genotypes (y axis) and all teosinte genotypes (y axis) was contrasted. The red data points indicate the 612 genes that are differentially expressed in maize relative to teosinte. The star-shaped data points indicate genes that were identified as domestication and/or improvement candidates by Hufford et al. (1). (D) Box plot is shown for the coefficient of variance for gene expression levels in all maize and teosinte genotypes analyzed for different subsets of genes. The genes are divided according to the depth of their homology. Genes with no homologs in maize or other species are classified as "NULL." Genes that have multiple family members in maize but are not detected in other species are classified as "Zea mays." Genes that have a sorghum homolog but no homologs in other grasses are classified as "Andropogoneae." Genes that have homologs in multiple grass species are classified as "Poaceae," and genes with homologs in other plant genomes are classified as "Magnoliophyta." (E) Box plot of gene expression variance is shown for genes that are located in syntenic (syn) and nonsyntenic (non_syn) genomic positions relative to other grass genomes. (F) Box plot shows gene expression variance for genes according to the gene family size in the maize genome.

1. Hufford MB, et al. (2012) Comparative population genomics of maize domestication and improvement. *Nat Genet*, 10.1038/ng.2309.



A



B

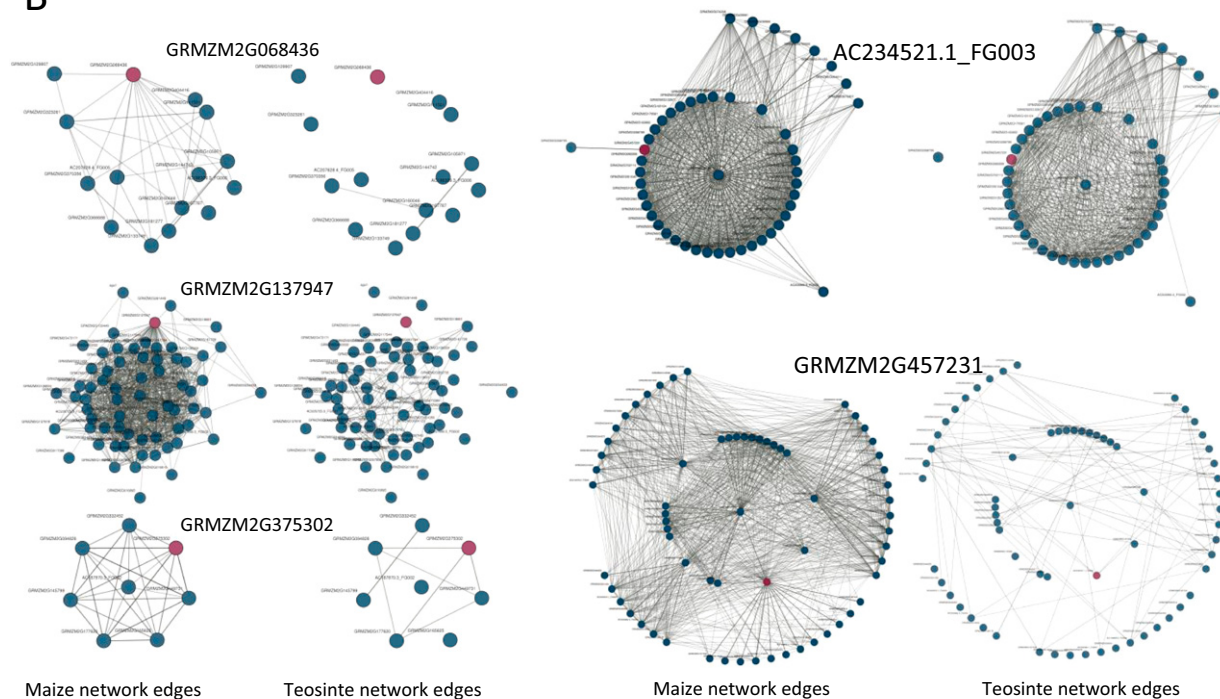


Fig. S4. (A) Partial conservation of coexpression networks following domestication is shown. Teosinte coexpression networks are shown for two highly connected genes. (Right) Edges that are maintained within maize coexpression networks are shown. Although the differentially expressed gene (red node) is highly connected in teosinte, most of these connections are lost in maize. However, some parts of the teosinte network are still conserved in maize. (B) Coexpression networks in maize and conserved edges in teosinte are shown. For the genes that were used in A and Fig. 3, we generated the maize coexpression network and then show the edges that are conserved in teosinte. This demonstrates that there are edges present in maize following domestication that were not apparent in teosinte.

1. Hufford MB, et al. (2012) Comparative population genomics of maize domestication and improvement. *Nat Genet*, 10.1038/ng.2309.

Table S1. List of genotypes and classifications

Genotype	Maize or teosinte	Cross-type	Group assignment	Tissue source	NAM?	Seed accession no.	Structure-based assignments	Hufford et al. (1)
A680	Maize	Inbred	SS	Individual		Ames 23503	SS	
B73	Maize	Inbred	SS	Pool	NAM	PI 550473	SS	Yes
B79	Maize	Inbred	MIX	Individual		PI 608766	NSS	
B84	Maize	Inbred	SS	Individual		PI 608767	SS	
B97	Maize	Inbred	NSS	Pool	NAM	PI 564682	NSS	Yes
CML103	Maize	Inbred	Trop	Pool	NAM	Ames 27081	Trop	Yes
CML228	Maize	Inbred	Trop	Pool	NAM	Ames 27088	Trop	Yes
CML247	Maize	Inbred	Trop	Pool	NAM	PI 595541	NSS	Yes
CML277	Maize	Inbred	Trop	Pool	NAM	PI 595550	Trop	Yes
CML322	Maize	Inbred	Trop	Pool	NAM	Ames 27096	Trop	Yes
CML333	Maize	Inbred	Trop	Pool	NAM	Ames 27101	Trop	Yes
CML52	Maize	Inbred	Trop	Pool	NAM	PI 595561	Trop	Yes
Hp301	Maize	Inbred	Pop	Pool	NAM	PI 587131	Trop	Yes
I205	Maize	Inbred	MIX	Individual		NSL 65871	NSS	
Il14H	Maize	Inbred	Sweet	Pool	NAM	Ames 27118	Other	Yes
Ki11	Maize	Inbred	Trop	Pool	NAM	Ames 27124	Trop	Yes
Ki3	Maize	Inbred	Trop	Pool	NAM	Ames 27123	Trop	Yes
Ky21	Maize	Inbred	NSS	Pol	NAM	Ames 27130	NSS	Yes
LH1	Maize	Inbred	PVP	Individual		PI 644101	Other	
LH82	Maize	Inbred	PVP	Individual		PI 601170	NSS	
M162W	Maize	Inbred	Trop	Pool	NAM	Ames 27134	Trop	Yes
M37W	Maize	Inbred	MIX	Pool	NAM	Ames 27133	Trop	Yes
Mo17	Maize	Inbred	NSS	Individual		PI 558532	NSS	Yes
Mo18W	Maize	Inbred	NSS	Pool	NAM	PI 550441	Trop	Yes
NC358	Maize	Inbred	Trop	Pool	NAM	Ames 27175	Trop	Yes
Oh43	Maize	Inbred	NSS	Pool	NAM	Ames 19288	Other	Yes
Oh7B	Maize	Inbred	NSS	Pool	NAM	Ames 19323	NSS	Yes
P39	Maize	Inbred	Sweet	Pool	NAM	Ames 28186	Other	Yes
PHG35	Maize	Inbred	PVP	Individual		PI 601008	NSS	
PHG39	Maize	Inbred	PVP	Individual		PI 600981	NSS	
PHG47	Maize	Inbred	PVP	Individual		PI 601320	NSS	
PHG84	Maize	Inbred	PVP	Individual		PI 601321	NSS	
PHJ40	Maize	Inbred	PVP	Individual		PI 601322	NSS	
PHZ51	Maize	Inbred	PVP	Individual		PI 601322	NSS	
Tx303	Maize	Inbred		Pool	NAM	Ames 19327	Trop	Yes
Tzi8	Maize	Inbred	Trop	Pool	NAM	PI 506246	Trop	Yes
W22	Maize	Inbred	NSS	Individual		NSL 30053	NSS	Yes
Wf9	Maize	Inbred	NSS	Individual		Ames 19293	NSS	
IA29P1	Teosinte	Outcrossed	TO	Individual		Ames 21809	TO	
IA29P2	Teosinte	Outcrossed	TO	Individual		Ames 21809	TO	
IA29P3	Teosinte	Outcrossed	TO	Individual		Ames 21809	TO	
IA29P4	Teosinte	Outcrossed	TO	Individual		Ames 21809	TO	
IA29P5	Teosinte	Outcrossed	TO	Individual		Ames 21809	TO	
IA29P6	Teosinte	Outcrossed	TO	Individual		Ames 21809	TO	
IA31P1	Teosinte	Outcrossed	TO	Individual		Ames 21810	TO	
IA31P2	Teosinte	Outcrossed	TO	Individual		Ames 21810	TO	
IA31P3	Teosinte	Outcrossed	TO	Individual		Ames 21810	TO	
IA31P4	Teosinte	Outcrossed	TO	Individual		Ames 21810	TO	
IA31P5	Teosinte	Outcrossed	TO	Individual		Ames 21810	TO	
IA31P6	Teosinte	Outcrossed	TO	Individual		Ames 21810	TO	
IA36P1	Teosinte	Outcrossed	TO	Individual		Ames 21814	TO	
IA36P2	Teosinte	Outcrossed	TO	Individual		Ames 21814	TO	
IA36P3	Teosinte	Outcrossed	TO	Individual		Ames 21814	TO	
IA36P5	Teosinte	Outcrossed	TO	Individual		Ames 21814	TO	
IA36P6	Teosinte	Outcrossed	TO	Individual		Ames 21814	TO	
TIL1	Teosinte	Inbred	TI	Individual		John Doebley*	TI	Yes
TIL11	Teosinte	Inbred	TI	Individual		John Doebley*	TI	Yes
TIL14	Teosinte	Inbred	TI	Individual		John Doebley*	TI	Yes
TIL15	Teosinte	Inbred	TI	Individual		John Doebley*	TI	Yes
TIL17	Teosinte	Inbred	TI	Individual		John Doebley*	TI	Yes
TIL6	Teosinte	Inbred	TI	Individual		John Doebley*	TI	Yes
TIL9	Teosinte	Inbred	TI	Individual		John Doebley*	TI	Yes

MI, mixed genetic background; NAM, nested association mapping parent; NSS, nonstiff stalk; Pop, popcorn; PVP, plant varietal protection; SS, stiff stalk; TI, teosinte inbred; TO, teosinte out-crossed; Trop, tropical.

*Gift from John Doebley (University of Wisconsin, Madison WI).

1. Hufford MB, et al. (2012) Comparative population genomics of maize domestication and improvement. *Nat Genet*, 10.1038/ng.2309.

Gene ID	Chr	M_avg	Po_avg	Pi_avg	DE	EC	Maize cluster	Teo cluster	EC	EC_zScore	Mdegree	Tdegree	md_teo_gen no.	McTeo z score	mdt_pval	Mclt z score	mcTeosinte_GO	ArabidopsisID	Short_description	P_inbl	P_out
GRMZM2G118173	1	826	1,747	319	No	No	103	776	0.125	-0.245	9	8	4	0.0665111	0	2.9871	Signal transduction	AT5G46730.1	Glycine-rich protein	0.18	
GRMZM2G469224	1	811	1,882	540	No	No	5	900	0.55	-0.762	110	56	4	0.7962056	0.055	1.7396		AT5G46730.1	Glycine-rich protein	0.29	
GRMZM2G444623	4	3,778	2,656	971	No	Yes	178	3,022	-0.335	-5.824	16	22	2					AT5G46730.1	Eukaryotic aspartyl protease family protein	0.37	
AC148152.3_FG008	2	5,185	5,799	2,220	No	No	166	2,558	0.387	0.037	12	45	2					AT3G06510.2	Glycosyl hydrolase superfamily protein	0.38	
GRMZM2G119638	3	2,542	3,889	1,495	No	No	5	5	0.283	0.377	0	11						AT4G15530.5	Pyruvate orthophosphate dikinase	0.38	
GRMZM2G097457	8	1,843	2,925	1,269	No	No	866	5	0.264	-0.483	1	70						AT1G59540.1	P-loop containing nucleoside triphosphate hydrolases	0.43	
GRMZM2G112745	4	2,124	3,103	1,502	No	No	2,587	993	0.277	-1.141	13	18	4	0.0901132	0	2.2822		AT1G59540.1	P-loop containing nucleoside triphosphate hydrolases	0.48	
GRMZM2G154508	8	7,413	9,717	4,768	No	No	7	11	0.268	0.068	23	53	108	-0.003823	0	1.2116		AT3G61880.2	Cytochrome p450 78a9	0.49	
GRMZM2G034471	2	2,224	3,965	1,985	No	No	5	5	0.091	-0.637	0	27						AT2G17560.1	High-mobility group B4	0.50	
GRMZM2G162284	2	2,474	7,309	3,674	Yes	No	2,284	5	0.346	-3.266	6	121	224	0.1216169	0	1.1298		AT3G62760.1	GST family protein	0.50	
GRMZM2G132093	10	774	394	1,238	No	No	77	10	0.307	-0.605	1	76	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT3G62760.1	GST family protein	3.14	
GRMZM2G056329	2	180	62	230	No	No	506	10	0.34	-0.921	5	122	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT4G20820.1	FAD-binding berberine family protein	3.72	
GRMZM2G080839	9	229	88	405	No	No	154	10	0.26	-1.49	5	119	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT1G73066.1	Leucine-rich repeat family protein	4.58	
GRMZM2G174128	5	186	71	335	No	No	350	10	0.008	-2.237	3	58	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT4G33420.1	Peroxidase superfamily protein	4.72	
AC205413.4_FG001	10	303	150	722	No	No	557	10	0.064	-0.442	4	106	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT3G56360.1		4.82	
GRMZM2G115839	1	153	67	330	No	No	2,672	10	0.165	-0.397	2	46	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT5G24090.1	Chitinase A	4.95	
GRMZM2G073114	5	604	209	1,050	No	No	2,911	422	0.342	0.611	1	42	5	0.0758775	0	5.2506				5.03	
GRMZM2G453805	3	6,035	1,505	7,717	Yes	Yes	136	10	0.169	-3.659	11	91	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT3G16120.1	Dynein light chain type 1 family protein	5.13	
GRMZM2G016435	3	547	129	733	Yes	No	994	10	0.394	-2.353	6	62	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region			5.70	

Gene ID	Chr	M_avg	Po_avg	Pi_avg	DE	EC	Maize cluster	Teo cluster	EC	EC_zscore	Mdegree	Tdegree	mcl_teo_gen no.	Mclteo z score	mclt_pval	Mclt z score	mdteosinte_GO	ArabidopsisID	Short_description	P_inh	P_out
GRMZM2G415529	7	465	191	1,127	No	No	48	10	0.265	-0.486	20	77	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT1G66950.1	Pleiotropic drug resistance 11	5.88	
GRMZM2G143153	3	773	309	1,826	No	No	154	10	0.129	-1.626	7	78	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region			5.91	
GRMZM2G135862	5	356	100	610	No	No	350	10	0.049	-2.706	1	66	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT4G36180.1	Leucine-rich receptor-like protein kinase family protein	6.07	
GRMZM2G023847	1	801	353	2,274	No	No	1,580	10	0.137	-0.679	5	67	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT3G60280.1	Uclacyanin 3	6.45	
GRMZM2G181227	10	2,716	771	4,999	No	No	459	10	0.296	-0.525	22	52	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT5G65940.1	β-Hydroxyisobutyryl-CoA hydrolase 1	6.48	
GRMZM2G039009	6	163	96	660	No	No	48	10	0.36	0.65	21	115	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT1G19640.1	Jasmonic acid carboxyl methyltransferase	6.87	
GRMZM2G089506	9	166	62	434	No	No	136	10	0.284	0.081	9	112	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT5G10770.1	Eukaryotic aspartyl protease family protein	7.03	
GRMZM2G176798	4	444	294	2,361	No	No	154	10	0.262	0.507	4	65	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT5G39110.1	RmlC-like cupins superfamily protein	8.02	
GRMZM2G131099	3	381	70	567	No	No	136	10	0.077	-2.438	23	114	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region			8.13	
GRMZM2G060630	4	135	232	1,913	Yes	Yes	1	5	-0.21	-6.06	190	14								8.25	
GRMZM2G174994	10	345	106	887	No	No	48	10	0.363	0.113	25	93	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region			8.37	
GRMZM2G016922	1	233	59	518	No	No	48	10	0.39	-1.148	18	96	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT1G79460.1	Terpenoid cyclases/protein prenyltransferases superfamily protein	8.77	
GRMZM2G057093	1	267	87	785	No	No	2,180	10	0.189	-0.561	2	69	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region			9.02	
GRMZM2G399530	10	330	110	1,090	No	No	135	10	0.213	-0.042	10	103	110	0.289182	0	3.1667	Molecular_function, response to biotic stimulus, extracellular region	AT5G24960.1	Cytochrome P450, family 71, subfamily A, polypeptide 14	9.89	

Table S2. Cont.

DE, differential expression; S, XXX.

