# Supporting Information

Barber et al. 10.1073/pnas.1202073109

## SI Materials and Methods

**Plant Materials and Phenotypic Measurements.** For the shoot apex sRNA sequencing experiment, 40 seeds of B73, Mo17, B73×Mo17, and Mo17×B73 were sown in separate flats consisting of 1:1:1 soil:peat:perlite mix and grown in a greenhouse (in Urbana, IL) under 16 h of light and 8 h of dark during the fall of 2007. Shoot apices were excised from 10 plants for each genotype 11 d after sowing (DAS). Maize seedlings were cut at their root nodes. Coleoptiles and all tissue from fully emerged leaves were removed by using a dissecting needle. Tissue enriched for leaf primordia and the shoot apex was isolated by cutting 10 mm above the base of the shoot. Shoot apices were pooled for each genotype and flash-frozen in liquid nitrogen. Additional shoot apex tissue used for quantification of *miR168* and *miR156* microRNA (miRNA) and *cinful* mRNA via quantitative real-time PCR (qRT-PCR) was collected from plants grown under the same conditions in the fall and winter of 2008 and 2009, but only three shoot apices were pooled per biological replicate. The shoot apex tissue used for additional sRNA sequencing was collected from B73 and Mo17 plants grown under the same green house conditions, but in winter 2011. In this experiment, 24 seeds were sown and three samples of three shoot apices were collected at the three- to four-leaf stage. The total RNA of the three samples was pooled in equivalent amounts such that the pool of total RNA used for the sRNA sequencing experiment represented tissue from nine plants.

For the developing ear sRNA sequencing experiment, tissue was collected from field-grown plots of B73, Mo17, and B73×Mo17 that were part of a larger yield-trial experiment, whereby inbreds and hybrids were grown in separate blocks that were split by nitrogen fertilizer treatment (summer 2009; Urbana, IL). Plots were either supplemented with recommended amounts of nitrogen for corn production in the Midwestern US (200 kg/ha) or not supplied additional nitrogen (low-N plots). In this experiment, 40 seeds were sown per 3.6-m row, and rows were spaced 76 cm apart. For each genotype, four rows were sown. The leaf number of the plants in the field was tracked by marking the leaves. Because hybrids mature faster than their parents, we relied on variation within the hybrid plot to sample hybrids at the same growth stage as their parents for the initial sequencing experiment. The top developing ear was excised from each genotype on the same day from plants at the V12 growth stage (i.e., at the time when the 12th leaf had fully expanded) for both the normal and low-N plots. Three ears were pooled for each genotype. Additional ear tissue used for *miR168* miRNA and *cinful* mRNA quantification was collected from field-grown plants under normal N conditions at the V10 and V11/V12 and V12/V13 growth stages during summer 2010. For the additional tissue, 25 seeds were sown per 5.6-m row, and rows were spaced 76 cm apart. For each genotype, 10 rows were sown. In the additional experiment, it should be noted that, 4 and 8 d after initial collection of ear tissue at the V10 stage, Mo17 was at the V11 and V12 stages, respectively, whereas B73 and B73×Mo17 are at the V12 and V13 stages. All ear tissue was collected in the morning between 9:00 AM and 11:00 AM.

The *mop1-1* loss-of-function allele has been previously described (1). The mutant allele was backcrossed for seven generations into the B73 inbred background and selfed for four generations to remove any residual heterozygosity. The mutant allele was backcrossed for five generations into the Mo17 inbred background parent and selfed for three generations. Heterozygous and homozygous *mop1-1* B73 and Mo17 mutant plants were differentiated using the following primers that assay for the presence of the *Mutator* insertion in exon 4 of *MOP1*: WT allele, mop1 forward, TTCGACGAGTTCCTGGACGC, mop1 reverse, GGGTGGTAGGTCACGTGGTA, expected amplicon size of 290 bp; mutant allele, mop1Mu forward, GCGCCCTGATGACCTACTAC, mop1Mu reverse, TGCGTCTCCAAAACAGAGAA, expected amplicon size of 170 bp. Homozygous *mop1-1* B73 and Mo17 mutant plants were selfed and crossed reciprocally, as were WT B73 and Mo17 parents (Urbana, IL). Parents and hybrids were planted in separate blocks during summer 2010 (Urbana, IL). WT and mutant parents and hybrids were planted in rows side by side in genotypic blocks. For each genotype, five rows of 25 seed were sown in 5.6-m rows that were spaced 76 cm apart. All rows were genotyped for the presence of the *mop1-1* allele. Phenotypic measures on representative individual plants taken from the middle of rows were collected for cob weight ($n$ = 4), height ($n$ = 5), and stover biomass ($n$ = 4). The dates for 50% silk and 50% anthesis were collected on individual rows. Total stover dry weight per plant for a plot was estimated as described in Uribelarrea et al. (2) with the following modifications. The ears were removed from the stover and saved for measurements, and the fresh stover was shredded with a Vermeer BC600 XL chipper without any partitioning of vegetative tissues.

**RNA Isolation.** RNA was extracted from the tissue by using TRIzol reagent according to the manufacturer's protocols (Invitrogen). Quantification and quality checks of total RNA were performed by A260/A280 spectrophotometry by using a Nanodrop ND-1000 (Thermo Fisher Scientific), gel electrophoresis, and total RNA Bioanalyzer chips (Agilent).

**sRNA Library Preparation and sRNA Sequencing.** sRNA libraries for the 2007 and 2011 shoot apex samples and the developing ear samples were prepared from 25 μg (2007) and 15 μg (2011) and 10 μg of total RNA. The 2007 shoot apex libraries were prepared by the Michael Smith Genome Sciences Centre and were sequenced on an Illumina/Solexa 1G Genome Analyzer (Illumina). The developing ear libraries were prepared by the high-throughput sequencing unit of the W. M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana–Champaign by using the Illumina sRNA kit (version 1.5) and were sequenced on an Illumina Genome Analyzer. We prepared the 2011 shoot apex libraries using the Illumina TruSeq Small RNA (sRNA) preparation kit. For all samples, total RNAs were separated on 15% TBE-Urea polyacrylamide gel (Invitrogen). Using a 10-bp ladder, the sRNA fraction representing 10 to 40 bp was cut from the gel and obtained via elution. sRNA libraries were constructed according to manufacturer's protocols (Illumina). For the 2007 shoot apex experiment, each library was sequenced by using one lane of a flow cell. Libraries were indexed with barcodes for the ear experiment (at high N, B73 V12-ATCG, Mo17 V12-ACGT, and B73×Mo17 V12- TCGA; at low N, B73 V12-TGAC, Mo17 V12-CTAG, and B73×Mo17 V12-CGTA), so multiple libraries could be sequenced per lane. The 2011 shoot apex samples were part of a larger sequencing experiment and sequenced in different lanes within the same flow cell on an Illumina HiSEq 2000 system. The libraries for this experiment were indexed by using barcodes (B73-ATCACG, Mo17-ATCACG).

**Processing of sRNA Sequencing Data.** The raw sRNA sequencing data were processed by using a combination of custom-designed perl scripts and scripts available in the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Only sequences containing the 5′ and 3′ adapters were retained, and both adapter sequences

were removed. For the developing ear experiment, the multiplexed libraries were split into individual libraries according to the barcode present in the sequence. Identical sequences were collapsed for each library. Sequences with ambiguous base-calls and with lengths falling outside of 18 to 29 nt were removed from the datasets. For each sequence, its abundance for a library was calculated by dividing its number of reads by the total number of raw reads generated for the library. For the ear datasets and the 2011 shoot apex dataset, only sRNAs sampled at an abundance of at least one read per million (rpm) in one of the ear libraries (B73, Mo17, B73×Mo17) were included in the dataset. For the 2007 shoot apex dataset, sequences with at least five reads in one of the libraries (B73, Mo17, B73×Mo17, Mo17×B73) were included in the dataset. For the 2007 shoot apex and the high-N ear experiments, sequences were combined across the libraries to identify an experiment-wide set of distinct siRNAs that was used for subsequent analyses. The abundance cutoffs were selected because they produced datasets with similar numbers and abundance of distinct siRNAs, correcting for the difference in sequencing depth between the experiments.

**Bioinformatic Analysis.** sRNAs were mapped to the B73 genome (version 4a.53; downloaded from http://ftp.maizesequence.org/current/assembly/, October 2009) and Mo17 whole-genome shotgun clones (454 paired and unpaired reads; downloaded from ftp://ftp.jgi-psf.org/pub/JGI_data/Zea_mays_Mo17/, January 2009) by using the short read aligner Novoalign (version 2.00.14; Novocraft). Only perfect matches along the entire sRNA sequence were considered mapped. sRNAs were annotated using the following databases: the maize miRNA hairpin sequences deposited in the miRBase miRNA registry (release 15, http://www.mirbase.org/) (3), the Rfam database (version 8.1, http://rfam.sanger.ac.uk/) (4, 5), the *Zea* Repeats database (http://plantrepeats.plantbiology.msu.edu) (6), and the *Arabidopsis* tRNA database (http://gtrnadb.ucsc.edu/Athal/). sRNAs were matched against these databases by using the PaTMaN DNA pattern matcher for short sequences (7). Except for miRNAs, a 1-bp mismatch was allowed for annotation purposes.

**Blocking of siRNAs to Generate of siRNA Clusters.** To identify siRNA clusters, we used a similar strategy to that used by Johnson et al. (8). Briefly, for both tissues, 21- to 24-nt sRNAs from all the genotypes not matching miRNAs, ribosomal DNA, or tRNA, and mapping to the Mo17 genome and the B73 genome 1 to 1,000 times were processed together. All the B73 locations were collected for this group of siRNAs and stored in a database. siRNAs within 100 bp of each other were placed into blocks referred to as siRNA clusters. The coordinates of the clusters are defined by the first and last siRNA of the overlapping sequences. siRNAs that map greater than 1,000 times to the B73 genome represented a very low percentage of total abundance of sRNAs for the experiments, so excluding them greatly reduced the number of siRNA clusters generated by our blocking approach. The abundance of an siRNA was distributed equally across all of its locations in the B73 genome before the blocking procedure and the summation of the abundances of all siRNAs present in a cluster. siRNA clusters with an abundance of at least 5 rpm in one of the genotypes were included in the analysis. siRNA clusters with less than 1 rpm were set to this abundance so the relative difference between parents could be calculated. Clusters were labeled according to which length of siRNA was in the majority (21, 22, and 24-nt) and by the genetic feature in which they are located in the B73 genome. Some clusters did not have a majority siRNA length, but clearly 21-nt and 22-nt siRNAs together made up the majority. These clusters were also labeled as 22-nt clusters because the number of 22-nt siRNAs was generally higher than the number of 21-nt siRNAs. The MIPS and MTEC repeat databases for the B73 genome (version 4a.53) were downloaded (http://ftp.maizesequence.org/current/repeats). The Filtered Gene Set GFF file for B73 (version 4a.53) was downloaded and parsed to only include the genes (http://ftp.maizesequence.org/current/filtered-set). A new GFF file was created by adding or subtracting 1,000 bp to the start and stop of each gene. To determine if the siRNA clusters overlapped with any B73 repeats or genes, the MIPS and MTEC repeat GFF files and the unmodified and modified Filtered Gene Set GFF files were intersected with siRNA cluster GFF files by using the open-source GFFintersect perl script (http://biowiki.org/GffTools/). Clusters that were completely located within a gene or within the 1,000 bp before and after the gene were characterized as gene regions. The remaining clusters were divided into repeats or intergenic regions based on whether they were completely located in repeat regions that were not located in or near genes.

**qRT-PCR.** Before reverse transcription (RT), total RNA was treated with Turbo DNase according to the manufacturer's directions to remove any genomic DNA contamination (Ambion). The RT reactions were performed using an MJ Research 225 Tetrad Thermal Cycler (Bio-Rad). The qRT-PCR reactions were performed using an MJ Research DNA Engine Opticon 2 Continuous Fluorescence Detection System.

For monitoring the expression of *cinful* and *GAPDH*, cDNA was reverse transcribed in a total volume of 20 μL. The RNA input reaction [0.8–1.0 μg of total RNA, 2 μL of oligo dT primer, dT23vn (NEB), and 1 μL of 10 mM dNTPs] was incubated at 65° for 5 min. The RT mixture [1 μL of M-MuLV reverse transcriptase (NEB), 1 μL of RNase inhibitor, human placenta (NEB), 2 μL of 10× M-MuLV reverse transcriptase reaction buffer (NEB), 2 μL of 0.1 M DTT (Invitrogen), and nuclease-free water] was added to the RNA input reaction and incubated at 42° for 60 min. The primers for monitoring *cinful* expression have been previously described (9). The primers used for monitoring *GAPDH* expression are: GAPDH forward, ACTGTGGATGTCTCGGTTGTTG; and GAPDH reverse, CCTCGGAAGCAGCCTTAATAGC. qRT-PCR for *cinful* and *GAPDH* was performed in 20 μL reactions consisting of 10 μL of 2× PerfeCTa SYBR Green FastMix (Quanta BioSciences) and 1 μL of both forward and reverse primers (10 μM). A total of 5 μL of 1:25 diluted cDNA was used for *GAPDH* qRT-PCR quantification, whereas 4 μL and 2 μL of cDNA was used for *cinful* qRT-PCR quantification in the shoot apex and the ear, respectively. qRT-PCR cycling parameters followed the manufacturer's recommendations and used an annealing temperature of 60°. Reactions were performed in duplicate. According to the $\Delta\Delta C_T$ method (10), *cinful* $C_T$ values were normalized using *GAPDH* $C_T$ values.

The accumulation of maize *miR156* and *miR168* were measured via qRT-PCR by using TaqMan miRNA assays (Applied Biosystems). We ordered a custom assay for maize *miR168*. We used a TaqMan miRNA assay designed for *Arabidopsis miR156a* to monitor expression levels of maize *miR156* because the species have the same mature miRNA sequence for these families. The RT reactions were performed using the TaqMan MicroRNA RT kit (Applied Biosystems) with the following modifications. For the shoot apex, the RT reaction consisted of 2 μL of total RNA diluted to 25 ng/μL, 3 μL of 5× *miR156* RT primer, 3 μL of 5× *miR168* RT primer, 2 μL of MultiScribe reverse transcriptase, 0.3 μL of 100 mM dNTPs, 1.5 μL of 10× RT buffer, 0.4 μL of RNase inhibitor, and 2.8 μL of nuclease-free water. The RT reaction for the developing ear contained 3 μL of 5× *miR166* RT primer in place of the *miR156* primer. Duplicate qRT-PCR reactions were performed according to the manufacturer's directions, using 9 μL and 3 μL of 1:15 diluted cDNA for *miR156* and *miR168*, respectively. According to the $\Delta\Delta C_T$ method (10), *miR168* $C_T$ values were normalized using *GAPDH* $C_T$ values, and *miR156* $C_T$ values were normalized using *miR168* $C_T$ values.

The molecular effect of the *mop1-1* mutation on the generation of 24-nt siRNAs was confirmed by using qRT-PCR to measure the accumulation of two 24-nt siRNAs (24-nt A, CGGCACGGTAGAATAAGCGGGCGG; 24-nt B, ACCCGGCACGGTAGAATAAGCGGG). We used the sRNA sequencing datasets generated by Nobuta et al. (11) to find these 24-nt siRNAs, which are reduced in abundance in the ear as a result of *mop1-1* (http://mpss.udel.edu/maize/index.php?menu=ftp.php). Primers for RT and qRT-PCR were designed for these siRNAs and *miR172* according to the sRNA qRT-PCR assay design shown by Yang et al. (12). To perform a multiplexed RT, equal volumes of 10 μM *miR172*, siRNA-1, and siRNA-4 RT primers were pooled. The RNA input reactions contained 4 μL of the primer mix, 4 μL of 10 mM dNTP mix, and 2 μL of total RNA diluted to 25 ng/μL. The RT and qRT-PCR reactions were performed as described by Varkonyi-Gasic et al. (13) by using the SuperScript III reverse transcriptase kit (Invitrogen) and 2× iQ SYBR Green Supermix (Bio-Rad). The qRT-PCR reactions contained 5 μL of 1:5 diluted cDNA and 1 μL of sRNA specific forward and universal reverse primers (10 μM), and were performed in triplicate. siRNA1 and siRNA4 $C_T$ levels were normalized by using *miR172* $C_T$ levels according to the $\Delta\Delta C_T$ method because the accumulation of *miR172* is not affected by the *mop1-1* mutation (14).

**Statistical Analysis.** Statistical tests were performed by using the SAS statistical software package (version 9.2; SAS). Correlation coefficients and significance values were calculated by using the CORR procedure. Wilcoxon rank-sum tests were performed by using the NPAIRWAY1 procedure. $\chi^2$ $2 \times 2$ contingency tests were performed by using the FREQ procedure. Two-sample *t* tests for a difference in means for agronomic traits between the *mop1* mutant and WT genotypes, and between the hybrid and midparent values, were performed by using the TTEST procedure. Elsewhere, basic data processes were performed in Excel workbooks (version 2007; Microsoft).


1. Alleman M, et al. (2006) An RNA-dependent RNA polymerase is required for paramutation in maize. *Nature* 442:295–298.
2. Uribelarrea M, Moose SP, Below FE (2007) Divergent selection for grain protein affects nitrogen use efficiency in maize hybrids. *Field Crops Res* 100:82–90.
3. Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ (2008) miRBase: Tools for microRNA genomics. *Nucleic Acids Res* 36(database issue):D154–D158.
4. Gardner PP, et al. (2009) Rfam: Updates to the RNA families database. *Nucleic Acids Res* 37(database issue):D136–D140.
5. Griffiths-Jones S (2005) Annotating non-coding RNAs with Rfam. *Curr Protoc Bioinformatics*, Chapter 12:Unit 12.
6. Ouyang S, Buell CR (2004) The TIGR Plant Repeat Databases: A collective resource for the identification of repetitive sequences in plants. *Nucleic Acids Res* 32(database issue):D360–D363.
7. Prüfer K, et al. (2008) PatMaN: Rapid alignment of short sequences to large databases. *Bioinformatics* 24:1530–1531.
8. Johnson C, et al. (2009) Clusters and superclusters of phased small RNAs in the developing inflorescence of rice. *Genome Res* 19:1429–1440.
9. Ohtsu K, et al. (2007) Global gene expression analysis of the shoot apical meristem of maize (Zea mays L.). *Plant J* 52:391–404.
10. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. *Methods* 25:402–408.
11. Nobuta K, et al. (2008) Distinct size distribution of endogeneous siRNAs in maize: Evidence from deep sequencing in the mop1-1 mutant. *Proc Natl Acad Sci USA* 105:14958–14963.
12. Yang H, et al. (2009) A novel real-time polymerase chain reaction method for high throughput quantification of small regulatory RNAs. *Plant Biotechnol J* 7:621–630.
13. Varkonyi-Gasic E, Wu R, Wood M, Walton EF, Hellens RP (2007) Protocol: A highly sensitive RT-PCR method for detection and quantification of microRNAs. *Plant Methods* 3:12.
14. Arteaga-Vazquez M, et al. (2010) RNA-mediated trans-communication can establish paramutation at the b1 locus in maize. *Proc Natl Acad Sci USA* 107:12986–12991.


| | % midparent heterosis | |
|---|---|---|
| B73xMo17 | Mean | SEM |
| Seedling biomass | 39.3 | 14.5 |
| $12^{th}$ leaf collar height | 59.4 | 10.2 |

**Fig. S1.** Heterosis is readily observed at the developmental stages investigated. Percent midparent heterosis observed for B73×Mo17 for seedling biomass and height to the sheath-blade junction (ligule) of the twelfth leaf. Reported measurements were taken on plants sampled for experiments.

**Fig. S2.** Functional classification, length distribution, and miRNA abundances for sRNA datasets. (*A*) Shown is the percentage of each dataset's total sRNA abundance accounted for by each functional class of sRNA for the shoot apex (*Left*) and the developing ear (*Right*). To classify sRNAs, sequences were first aligned to the maize miRNA database and the *Zea*, Rfam, and *Arabdopsis* tRNA repeats databases (*Materials and Methods*). sRNAs that did not fall into the miRNA, mite, transposon, retrotransposon, ribosomal DNA, and tRNA categories were classified based on their number of locations in the B73 genome or if they only perfectly matched the Mo17 genome. (*B*) Comparison of miRNA family abundance across each experiment and between the genotypes in each experiment. The total abundance column displays the abundance of 20- to 22-nt sRNAs matching the mature miRNA sequence for each miRNA family relative to each other in the shoot apex (*Left*) and the ear (*Right*). For the miRNA families, the abundance of each genotype is displayed relative to the other genotypes. (*C*) Percentage of genotypes' total sRNA abundance partitioned by length for the shoot apex (*Left*) and the developing ear (*Right*).

B.

| Genotype | First leaf glossy: Mean Node position | SEM |
|---|---|---|
| B73 | 6.7 | 0.2 |
| Mo17 | 5.4 | 0.2 |
| B73xMo17 | 6.2 | 0.2 |

**Fig. S3.** qRT-PCR of *microRNA156*, *microRNA168*, and *cinful* in the shoot apex and developing ear. (*A*) B73×Mo17 does not have elevated *microRNA168* levels compared with its parents in the seedling shoot apex or the developing ear. Shown are ΔΔCt values for *miR168* as the target gene and *GAPDH* as the reference for the shoot apex (*Left*) and developing ear (*Right*). Expression values are relative to B73 samples at the three-leaf stage or the V10 growth stage. Error bars represent SEM of the ΔΔCt values obtained from averaging three biological replicates. (*B* and *C*) B73×Mo17 and Mo17 seedlings similarly proceed through phase change earlier than B73 seedlings. (*B*) Shown are the node positions for the first leaf glossy for B73, Mo17, and B73xMo17 plants. Phenotypes were determined from six independent observations of at least three plants from field grown plots over four summers. (*C*) Shown are ΔΔCt values for *miR156* as the target gene and *miR168* as the reference for the shoot apex. Expression values are relative to B73 samples at the three-leaf stage. Error bars represent ±SEM of the mean of the ΔΔCt values obtained from averaging three biological replicates. (*D*) B73 seedling shoot apices and developing ears have a higher level of *cinful* expression than those of Mo17. Shown are ΔΔCt values for *cinful* as the target gene and *GAPDH* as the reference for the shoot apex (*Left*) and developing ear (*Right*). Expression values are relative to B73 samples at the five-leaf stage and the V10 growth stage. Error bars represent SEM of the ΔΔCt values obtained from averaging three biological replicates.

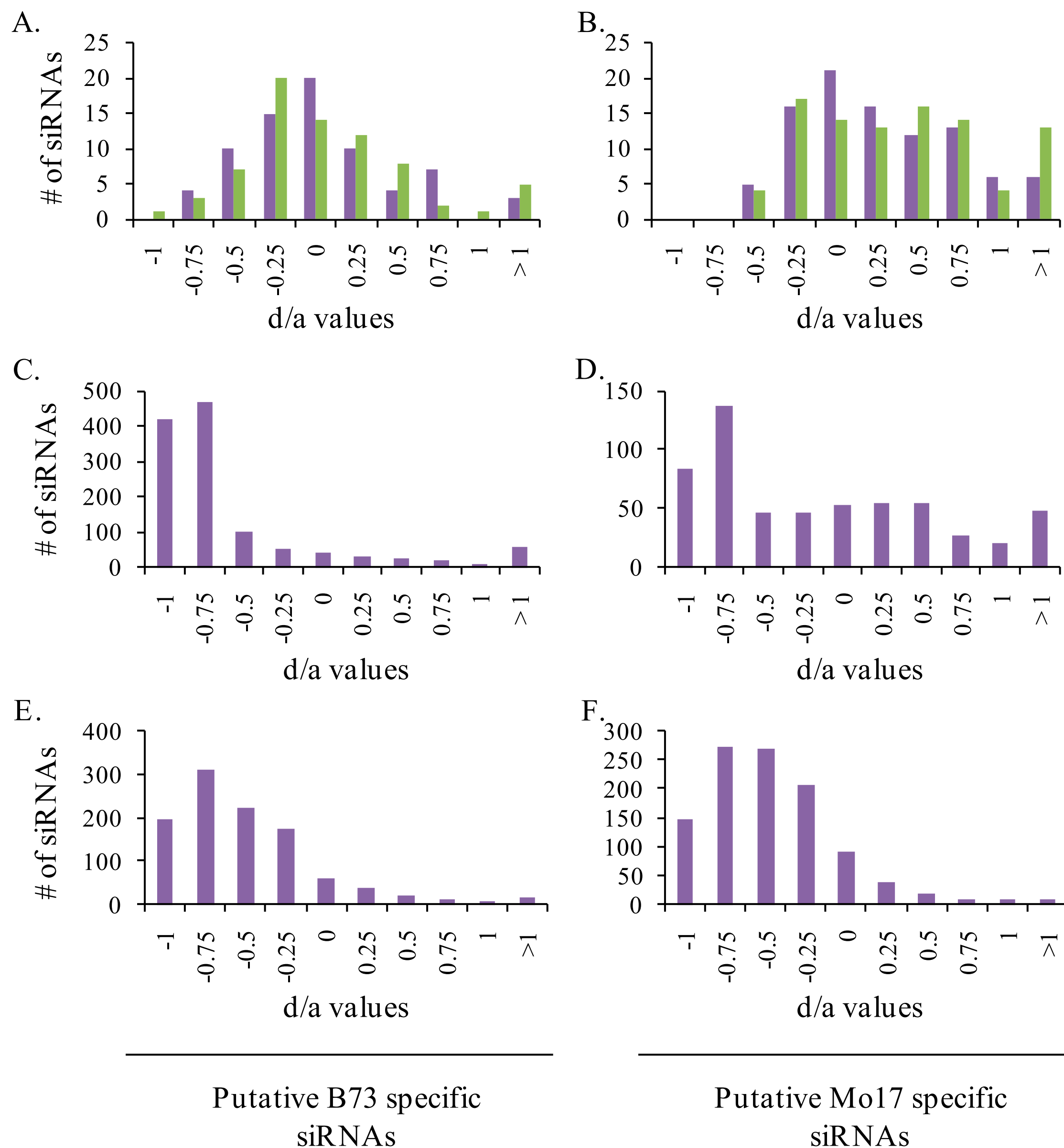


**Fig. S4.** Analysis of inheritance of putative parental specific siRNAs shows these siRNAs are inherited in an additive fashion in the shoot apex and tend to be inherited at levels below the midparent in the ear. Shown are the distributions of d/a values, the hybrid deviation from midparent abundance relative to difference between parental abundances, for putative B73- and Mo17-specific siRNAs for the shoot apex (*A* and *B*) and developing ear grown with (*C* and *D*) or without (*E* and *F*) supplemental nitrogen. Purple and green bars are d/a values for B73×Mo17 and Mo17xB73, respectively. siRNAs with an abundance of at least 5 rpm in B73 or Mo17 were included in this analysis.

**Fig. S5.** For the developing ear samples at low nitrogen, the abundance of 24-nt siRNA clusters in hybrids trends to below midparent levels as the difference between the parental abundances increases. Shown are the 22-nt and 24-nt siRNA clusters with at least 5 rpm-repnorm in the developing ear at low nitrogen ($n$ = 6,044). Clusters are arranged in ascending order of parental fold change. (*A*) Classification of clusters based on type and genetic feature. (*B*) Degree of parental difference for siRNA clusters (log10 values of high parent abundance divided by low parent abundance). Clusters below the horizontal blue line have parental differences that fall within the top 10% of the values for all of the clusters (threshold, 9.8-fold). (*C*) Deviation from midparent values for siRNA clusters (log2 values of F1 abundance divided by midparent abundance).

**Fig. S6.** Parental differences in retrotransposon siRNA activity are driven by 21-nt and 22-nt siRNAs. sRNA profiles are displayed for 2011 seedling shoot apex experiment (*Left*) and developing ear at low nitrogen experiment (*Right*). The total abundance column displays the relative abundance of 21-, 22-, and 24-nt siRNAs matching maize characterized retrotransposon families (at most 1-bp mismatch). For each retrotransposon family, abundance is partitioned by genotype and siRNA length.

**Table S1. Statistics of sRNA sequencing and summary of distinct sRNA datasets**

| Library | Raw reads | After processing data to generate distinct sRNA dataset | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | No. distinct sequences | Raw reads | Sequences mapping to both genomes, % | Sequences mapping to B73 genome, % | Sequences only mapping to B73 genome, % | Sequences mapping to Mo17 genome, % | Sequences only mapping to Mo17 genome, % |
| 2007 shoot apex experiment | | | | | | | | |
| B73 | 8,635,395 | 75,048 | 747,838 | 85 | 96 | 11 | 89 | 4 |
| Mo17 | 6,132,828 | 63,132 | 483,558 | 84 | 86 | 2 | 98 | 14 |
| B73×Mo17 | 8,526,014 | 88,731 | 917,954 | 80 | 88 | 8 | 92 | 12 |
| Mo17xB73 | 8,539,768 | 90,120 | 985,369 | 80 | 88 | 8 | 92 | 12 |
| Combined dataset | 31,834,005 | 95,665 | 3,134,719 | 79 | 88 | 9 | 91 | 12 |
| 2011 shoot apex experiment | | | | | | | | |
| B73 | 36,436,365 | 76,444 | 11,323,869 | 78 | 93 | 16 | 81 | 6 |
| Mo17 | 36,449,268 | 74,544 | 10,658,233 | 78 | 84 | 6 | 94 | 16 |
| Combined dataset | 72,885,633 | 85,814 | 21,982,102 | 72 | 86 | 14 | 86 | 14 |
| High-N developing ear experiment | | | | | | | | |
| B73 | 4,775,285 | 82,379 | 979,092 | 75 | 99 | 24 | 76 | 1 |
| Mo17 | 6,410,589 | 71,382 | 1,127,950 | 80 | 83 | 3 | 97 | 17 |
| B73×Mo17 | 6,146,647 | 81,695 | 1,025,760 | 74 | 88 | 15 | 85 | 12 |
| Combined dataset | 17,332,521 | 118,625 | 3,132,802 | 71 | 89 | 18 | 82 | 11 |
| Low-N developing ear experiment | | | | | | | | |
| B73 | 3,840,712 | 85,965 | 792,687 | 75 | 99 | 23 | 76 | 1 |
| Mo17 | 6,016,372 | 79,047 | 1,120,157 | 79 | 83 | 3 | 97 | 17 |
| B73×Mo17 | 5,677,392 | 97,005 | 1,056,852 | 72 | 89 | 19 | 84 | 11 |
| Combined dataset | 15,534,476 | 125,712 | 2,969,696 | 70 | 88 | 18 | 82 | 12 |

sRNA, small RNA.

**Table S2. Description of siRNA clusters found to have same parental differences in top 10% tails of siRNA clusters for all four sequencing datasets**

| Genomic region containing siRNA clusters | | Type of clusters, nt | High parent | Abundance in 2007 shoot apex libraries | | | | Abundance in 2011 shoot apex libraries | | Abundance in high-N ear libraries | | | Abundance in low-N ear libraries | | | Annotation of siRNA region |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chromosome | Start-stop | | | B73 | Mo17 | B73×Mo17 | Mo17xB73 | B73 | Mo17 | B73 | Mo17 | B73×Mo17 | B73 | Mo17 | B73×Mo17 | |
| 1 | 29866587–29869503 | 22 | Mo17 | 45 | 0 | 23 | 26 | 24 | 0 | 20 | 0 | 8 | 22 | 0 | 6 | ji retrotransposon |
| 2 | 6209266–6210574 | 22 | Mo17 | 1 | 28 | 19 | 23 | 4 | 66 | 7 | 69 | 49 | 1 | 67 | 48 | ji retrotransposon |
| 2 | 71020628–71023520 | 22 | B73 | 14 | 0 | 9 | 9 | 8 | 0 | 11 | 0 | 8 | 52 | 0 | 18 | cinful retrotransposon |
| 4 | 96384358–96387810 | 22 | B73 | 19 | 0 | 10 | 11 | 15 | 0 | 11 | 0 | 4 | 16 | 0 | 6 | cinful-zeon retrotransposon |
| 5 | 52441250–52443143 | 22 | Mo17 | 3 | 95 | 57 | 74 | 24 | 213 | 7 | 74 | 55 | 0 | 20 | 4 | ji retrotransposon |
| 5 | 206789580–206789810 | 22 | Mo17 | 3 | 48 | 42 | 54 | 1 | 151 | 7 | 78 | 61 | 0 | 46 | 17 | ji retrotransposon |
| 9 | 118303216–118304068 | 22 | Mo17 | 1 | 21 | 13 | 16 | 3 | 61 | 4 | 60 | 56 | 3 | 65 | 35 | ji retrotransposon |
| 10 | 135687786–135688796 | 24 | B73 | 30 | 0 | 16 | 26 | 26 | 0 | 56 | 0 | 32 | 54 | 0 | 18 | Exon of gene GRMZM2G454425 |
| 10 | 143818843–143822833 | 22 | B73 | 244 | 0 | 123 | 131 | 988 | 1 | 427 | 0 | 217 | 707 | 0 | 151 | Victim retrotransposon |
| Unknown | 4005307–4005660 | 22 | B73 | 32 | 0 | 16 | 16 | 80 | 0 | 36 | 0 | 12 | 40 | 0 | 11 | None |

**Table S3. Mean agronomic trait values for *mop1-1* and WT B73, Mo17, and their reciprocal hybrids**

| | Agronomic trait | | | | |
|---|---|---|---|---|---|
| Genotype | Height, cm | 50% Anther, d | 50% Silk, d | Cob weight, g | Stover biomass, g |
| B73 | 201.5 | 72.60 | 75.60 | 19.3 | 92.4 |
| B73 mop1-1/mop1-1 | 182.3* | 80.6† | 84.8† | 5.1† | 67.3‡ |
| Mo17 | 210.9 | 70.60 | 76.20 | 12.2 | 86.5 |
| Mo17 mop1-1/mop1-1 | 196.1§ | 72.2* | 84.6† | 2.4† | 66‡ |
| B73 × Mo17 | 269 | 67.60 | 70.40 | 23.3 | 130.1 |
| B73 × Mo17 mop1-1/mop1-1 | 250.6* | 69* | 79.4† | 14.5§ | 128.1 |
| Mo17 × B73 | 273.8 | 67.80 | 71.20 | 21.4 | 125.9 |
| Mo17 × B73 mop1-1/mop1-1 | 257.9* | 68.8* | 79.2† | 15.1§ | 123.2 |

*$P < 0.01$, †$P < 0.0001$, ‡$P < 0.1$, and §$P < 0.001$ (two-sample *t* tests of equal means).

# Other Supporting Information Files

Dataset S1 (XLSX)