Extensive intraspecific gene order and gene structural variations between Mo17 and other maize genomes

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Maize is an important crop with a high level of genome diversity and heterosis. The genome sequence of a typical female line, B73, was previously released. Here, we report a de novo genome assembly of a corresponding male representative line, Mo17. More than 96.4% of the 2,183 Mb assembled genome can be accounted for by 362 scaffolds in ten pseudochromosomes with 38,620 annotated protein-coding genes. Comparative analysis revealed large gene-order and gene structural variations: approximately 10% of the annotated genes were mutually nonsyntenic, and more than 20% of the predicted genes had either large-effect mutations or large structural variations, which might cause considerable protein divergence between the two inbred lines. Our study provides a high-quality reference-genome sequence of an important maize germplasm, and the intraspecific gene order and gene structural variations identified should have implications for heterosis and genome evolution.

Maize (Zea mays) is a classical genetic model and an important crop worldwide. The maize genome exhibits high levels of genetic diversity among different inbred lines1–6. Owing to the high level of intraspecific genome diversity, hybrid maize resulting from crosses between lines from different heterotic groups shows extremely high levels of hybrid vigor. Consequently, the adoption of hybrid maize has grown rapidly since its introduction nearly a century ago7. Currently, most modern maize varieties are hybrids. Reid yellow dent (represented by inbred B73) and Lancaster (represented by inbred Mo17) are the two best-known maize variety groups. The hybrid generated by crossing B73 with Mo17 had long been the most commonly grown hybrid in America and other countries8, and the derived materials are still widely used in many maize breeding programs. B73/Mo17 is therefore the most common pair of maize inbred lines in many genetic and molecular studies, such as map-based cloning9 or exploring the molecular basis of heterosis10 and genetic imprinting11–13. The intermated population of B73 and Mo17 is the most prominent maize genetic-mapping population14. The release of the draft genome assembly of inbred line B73 in 2009 (ref. 9) was an important milestone in the maize community15. The draft genome sequences of the maize PH207 inbred line assembled from short reads have also been reported16. The precise genomic arrangement of maize has recently been demonstrated to be resolvable through assembly of PacBio long reads17, which are up to 40–60 kb long, albeit with a relatively high error rate18. Long-read assembly has been used to generate several other plant and animal genomes19–22. Recently, a high-quality whole-genome assembly of B73 (RefGen_v4) has been generated through single-molecule technologies and the BioNano Irys system23, thus resulting in major improvements relative to the earlier assembly.

Here, we report the assembly of a high-quality Mo17 reference genome through single-molecule sequencing and BioNano optical-mapping technologies. The generation of an additional reference genome provides an unprecedented opportunity for extensive comparison of intraspecific genome diversity in maize. By aligning the B73 and Mo17 genomes, we found 9,867,466 SNPs; 1,422,446 small insertions/deletions (indels, length shorter than 100 bp); and more than 25 MB of presence/absence-variation (PAV, length longer than 500 bp) sequences between the two representative maize genomes. Notably, our comparative genomics analysis uncovered extensive intraspecific gene-order variation: approximately 10% of genes were mutually nonsyntenic between B73 and Mo17. In addition, more than 20% of the annotated genes had large-effect mutations or large structural variations in B73 compared with Mo17. These large gene-order and gene structural variations were also observed in a comparison of the PH207 genome with the B73 and Mo17 genomes.

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1289
Results
Genome sequencing and assembly. We sequenced and assembled the genome of Mo17 through a combination of three technologies: single-molecule real time (SMRT) sequencing with the PacBio Sequel platform, paired-end sequencing with the Illumina HiSeq platform and optical genome mapping with the BioNano Genomics Irys System (Methods). The initial assembly of 24.11 million PacBio long reads (200.8 Gb in total), representing ~90× sequencing coverage of the Mo17 genome, resulted in a 2,148-Mb assembly with a contig N50 size of 1.48 Mb (Table 1 and Supplementary Tables 1 and 2). The assembled contigs were polished with Pilon24 with 251.8-Gb high-quality paired-end PCR-free reads, then scaffolded by optical maps assembled with 267.7-Gb BioNano molecules. The final assembly contains 2,560 scaffolds with a scaffold N50 size of 10.2 Mb (Table 1 and Supplementary Table 2). The total assembly size of the Mo17 genome is 2,183 Mb, a genome size similar to that of the recently updated B73 genome (2,106 Mb)23. Approximately 97.2% (1,399 of 1,440) of embryophyta genes were detected in our assembly according to BUSCO25, a percentage similar to that for the B73 genome (97.3%), thus indicating the near completeness of our assembly. To evaluate the quality of the assembled Mo17 genome, we downloaded nine previously published Mo17 bacterial artificial chromosome (BAC) sequences from GenBank and aligned them to the scaffolds with BLAT26. All nine BAC sequences were covered by a single scaffold with high consistency and coverage (Supplementary Table 3), thus indicating high quality of the assembled genome. With a high-density genetic map containing approximately 4.4 million genotype-by-sequencing (GBS) tags27, we anchored and oriented 362 scaffolds onto ten pseudochromosomes, which account for 96.42% (2,104 Mb) of the genome assembly (Supplementary Table 4). The alignment of the Mo17 and B73 genomes with the anchored GBS tags27 showed high consistency with respect to the position and orientation of the anchored scaffolds (Supplementary Fig. 1).

Genome annotation. Repetitive elements, major components of complex genomes, are widely dispersed throughout the genome and have multiple roles in driving genome evolution28. In total, approximately 83.83% of the Mo17 assembly sequences were annotated as repetitive elements, including retrotransposons (75.24%), DNA transposons (6.12%) and unclassified elements (1.72%) (Supplementary Table 5). The families of Gypsy and Copia retrotransposons represented approximately 48.63% and 25.57% of the Mo17 assembly sequences, respectively. The composition of the different classes of repetitive DNA in Mo17 was highly similar to that in the B73 and PH207 genomes (Supplementary Fig. 1).

To examine transposon activity, we identified a total of 73,459, 74,160 and 50,402 high-confidence long terminal repeat (LTR) retrotransposons in the B73, Mo17 and PH207 genomes, respectively. The expansion of LTR retrotransposons in maize occurred mainly within the past 1 million years in both the B73 and Mo17 genomes (Supplementary Fig. 2), in agreement with previous estimates based on analysis of selected regions of the maize genome6,11. A relatively lower percentage of young LTR retrotransposons was seen in the PH207 genome assembly, because highly similar copies were collapsed when assembled from short reads (Supplementary Fig. 2). Compared with the older LTR retrotransposons, which were more abundant in pericentromeric regions, the younger LTR retrotransposons were enriched in euchromatic regions (Supplementary Fig. 3), in agreement with findings from previous studies1,2,8.

To annotate the protein-coding genes in the Mo17 genome, we combined results obtained from protein-homology-based prediction, RNA-seq-based prediction and ab initio prediction (Methods), an approach similar to that used for the annotation of the B73 (refs 23,35) and PH207 (ref. 16) genomes. In total, 38,620 high-confidence protein-coding genes were predicted in the Mo17 genome, and 55% of the exons of the predicted genes were supported by RNA-seq data from five different tissues with at least 90% coverage (Supplementary Tables 6 and 7). In total, 37,830 (97.95%) of the Mo17 predicted genes were allocated in ten pseudochromosomes (Supplementary Table 4). Protein-coding genes were primarily within chromosome arms and were inversely correlated with transposable-element density (Fig. 1).

Global genome comparison of B73, Mo17 and PH207. When the pseudochromosomes of Mo17 were aligned to the pseudochromosomes of B73 (ref. 15), approximately 61.18% of the Mo17 genome sequence matched in one-to-one syntenic blocks with 61.13% of the B73 genome sequence (Supplementary Fig. 4 and Supplementary Table 8). The genome-wide proportion of the regions that nearly matched between B73 and Mo17 was slightly higher than that estimated from a previous analysis of sequenced BAC clones of four selected regions1. The nonsyntenic sequences between the two genomes were mostly transposable elements, and the remainder comprised dispersed genes and inbred line-specific low-copy sequences. Similarly, we found 1,071.7 Mb (50.93%) of the Mo17 genome sequence and 1,101.7 Mb (52.3%) of the B73 genome sequence matching in syntenic blocks with 1,071.6 Mb (52.01%) and 1,101.4 Mb (53.46%) of the current PH207 genome sequences, respectively (Supplementary Table 8).

By comparing the two genomes, we identified 12,936 B73-specific genomic segments (12.96 Mb in total) and 12,939 Mo17-specific genomic segments (12.2 Mb in total) longer than 500 bp. Most (98.7%) of these PAV sequences were shorter than 5 kb (Supplementary Fig. 5). We found 200 and 126 PAV sequences that were longer than 5 kb in B73 and Mo17, respectively. These PAV sequences were unevenly distributed across the genome (Fig. 1), and some were located in clusters (Supplementary Table 9). The longest PAV sequence segment was a 2.9-Mb B73-specific segment containing 66 predicted genes, found from 22.5 Mb to 25.4 Mb on chromosome 6. The length of this PAV segment was slightly longer than previously reported1. The longest Mo17-specific segment was a 2.5-Mb segment on chromosome 6 from 64.0 Mb to 66.5 Mb. This Mo17-specific segment was close to the centromere and contained only ten predicted genes. In addition, we found two Mo17-specific segments located close together on chromosome 2, with lengths of 752.6 kb (chromosome 2: 235809501–236562100) and 635.2 kb (chromosome 2: 237529501–238164700), containing 20 and 23 predicted genes, respectively (Supplementary Table 9). With the criterion requiring at least 75% of coding sequences to overlap with PAV sequences, and validation through alignment of resequencing reads, we identified 72 B73-specific PAV genes and 50 Mo17-specific PAV genes. The number of PAV genes identified with this stringent criterion was smaller than the earlier estimation using only

### Table 1: Global statistics for the Mo17 genome assembly

|                        | PacBio assembly | BioNano assembly | Hybrid assembly | Pseudomolecule |
|------------------------|-----------------|------------------|-----------------|----------------|
| Total length of assembly (Mb) | 2,147.54        | 2,244.58         | 2,182.62        | 2,104.47       |
| N50 size (Mb)          | 1.48            | 1.41             | 10.2            | –              |
| Longest length (Mb)    | 7.26            | 9.35             | 32.18           | –              |
| Number of sequences    | 4,257           | 2,473            | 2,560           | 10             |

Table 1: Global statistics for the Mo17 genome assembly.
We also identified 22 PH207-specific PAV genes, as compared with B73, and 75 PH207-specific PAV genes, as compared with Mo17, by using the same method (Supplementary Table 10); only four genes overlapped between the two sets. We found that only ~25% of these PAV genes in the B73, Mo17 and PH207 genomes have likely orthologs in sorghum (Supplementary Table 10). To further trace the origin of these PAV genes, we aligned resequencing reads of 19 wild relatives, 23 landraces and 60 modern inbred lines from maize Hapmap2 (ref. 36) projects to the B73, Mo17 and PH207 genomes. Closely related homologs of more than 95% of these PAV genes were detected in at least one of the wild relatives, thus indicating that most of these PAV genes might have already existed in their direct ancestors (Supplementary Table 10). In summary, most of the PAV genes are present in the wild relatives of maize. These PAV genes might have arisen during the process of rediploidization of maize ancestors, which occurred before the completion of maize domestication but after the divergence of sorghum and maize. Thus, these PAV genes are now dispersed in landraces and modern maize lines (Supplementary Fig. 6).

A total of 9,867,466 SNPs and 1,422,446 indels were identified in the aligned syntenic blocks between the B73 and Mo17 genomes, with an average of 7.66 SNPs and 1.11 indels per kilobase (Supplementary Table 11). The distribution of SNPs and indels was positively correlated (Pearson’s correlation $R = 0.76$, $P < 8.7 \times 10^{-17}$; Fig. 1). Compared with indels genome wide, indels with multiples of 3 bp in length were more abundant in coding regions (Supplementary Fig. 7). We also identified 8,598,810 SNPs and 1,190,826 indels in the aligned syntenic sequences between the Mo17 and PH207 genomes, and 8,290,900 SNPs and 1,078,722 indels in the aligned syntenic sequences between the B73 and PH207 genomes (Supplementary Table 8).

Maize is an ancient tetraploid, and its two subgenomes have undergone extensive gene fractionation. Using fractionation bias estimation with sorghum as a reference, we found that the sub-genome organizations of B73 and Mo17 were nearly identical (Supplementary Fig. 8), thus indicating that B73 and Mo17 share the same tetraploidization and a large part of the subsequent fractionation events. Fractionation of genes was similarly biased toward subgenome 1 in both B73 (23,029 and 14,877 genes for subgenomes Mo17 Chr10
B73 Chr10
Mo17 Chr9
Mo17 Chr8
Mo17 Chr7
Mo17 Chr6
Mo17 Chr5
Mo17 Chr4
Mo17 Chr3
Mo17 Chr2
Mo17 Chr1
B73 Chr9
B73 Chr8
B73 Chr7
B73 Chr6
B73Chr5
B73 Chr4
B73 Chr3
B73 Chr2
B73 Chr1
Subgenome
Repeat density (%)
Gene density (%)
SNPs (number/Mb)
PAV sequence length (kb)
Indels (number/Mb)
Best-hit gene pairs
0 2,500
0 150
0 40
0 15,000
0.5 20
f
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Fig. 2 | Evolutionary constraints among B73, Mo17 and PH207 genomes. a, Ks distribution of each two of these three genomes. b, Ka/Ks distribution of each two of these three genomes.

1 and 2, respectively) and Mo17 (22,471 and 14,366 genes for subgenomes 1 and 2, respectively), and subgenome 1 (B73, 1,206 Mb; Mo17, 1,205 Mb) had an approximately ~1.62-fold-longer physical length than that of subgenome 2 (B73, 740 Mb; Mo17, 745 Mb; Fig. 1 and Supplementary Fig. 8).

To detect genes that might be under selection, we first calculated the neutral mutation rate (Ks) between orthologous genes for each pair between any two genomes of B73, Mo17 and PH207. We found two peaks in the Ks distribution: one corresponded to a group of genes that might derive from recent genetic exchanges (Ks < 0.0028), and another represented most of the remaining genes that may have diverged from the common ancestors of maize approximately 2.1 Ma (Ks ~0.025) (Fig. 2a). We then calculated the Ka/Ks ratio for genes with Ks between 0.0028 and 0.25 (Fig. 2b).

As expected, the Ka/Ks ratios for these genes were highly skewed toward zero, because most nonsynonymous mutations were deleterious and experienced strong purifying selection. Approximately 7,000 genes in each of the three genomes were identified to be likely to be evolutionary constrained (Ka/Ks < 0.1). In contrast, relatively few genes (>1,000) were detected to be under positive selection (Ka/Ks > 1).

Extensive gene-order and structural variations. Comparative analysis revealed 33,681 B73 and 33,597 Mo17 genes with corresponding orthologous genes or gene fragments in their syntenic regions. However, we found 5,105 B73 genes and 4,008 Mo17 genes that were nonsyntenic, because no homologous genes or gene fragments were found within 10 Mb of their corresponding positions in their respective counterpart genome (Table 2); these nonsyntenic genes accounted for 13.16% and 10.66% of the total analyzed genes in the B73 and Mo17 genomes, respectively. Similarly, 3,284 (9.02%) and 4,472 (12.27%) PH207 genes were defined as nonsyntenic genes when compared with B73 and Mo17 genomes, respectively (Supplementary Tables 12 and 13). Notably, 2,112 PH207 genes were nonsyntenic with both the B73 and Mo17 genomes.

Among the syntenic genes in B73 and Mo17 were 12,167 B73 and 12,674 Mo17 genes without any amino acid changes, and approximately 80% and 57% of genes showed no variation in coding sequences (CDSs) and gene bodies (CDS and intron), respectively (Table 2). We identified 2,498 B73 and 2,458 Mo17 highly conserved genes with no genetic variation in their entire genic regions, including within 2 Kb upstream and downstream (Table 2). There were 15,955 B73 and 15,512 Mo17 genes with only missense mutations and/or nonframeshift indels; those genes, together with the genes without amino acid changes, were classified as structurally conserved genes. These structurally conserved genes accounted for approximately 84% of the syntenic genes on the basis of either the B73 or the Mo17 gene annotation (Table 2). However, approximately 12% of the syntenic genes (3,947 in B73; 4,020 in Mo17) had large-effect mutations such as start- or stop-codon mutations, splice-donor or splice-acceptor mutations, frameshift mutations or premature-stop-codon mutations (Table 2). In addition, 1,612 B73 and 1,391 Mo17 genes had large structural variations, as compared with their corresponding syntenic genes in the Mo17 and B73 genomes, respectively (Table 2).

We then conducted additional analysis of the nonsyntenic genes between B73 and Mo17. On the basis of the analysis of a simple best hit in the counterpart genome, we classified 1,534 B73 and 1,216 Mo17 nonsyntenic genes as structurally conserved (Table 2). 1,387 B73 and 977 Mo17 nonsyntenic genes had large-effect mutations. In addition, 2,112 B73 and 1,765 Mo17 nonsyntenic genes had large structural variations, and 87 B73 genes and 253 Mo17 genes had no homologs identified in the Mo17 or B73 genome. Clustering of all annotated B73 and Mo17 genes revealed 320 B73-specific gene families (830 total gene members) as compared with Mo17, and 170 Mo17-specific gene families (578 gene members) as compared with B73. Among the 5,105 nonsyntenic genes in B73, 4,285 genes belonged to 2,114 gene families, including 294 B73-specific gene families (465 gene members). Among the 4,008 nonsyntenic genes in the Mo17 genome, 3,225 genes belonged to 1,631 gene families, including 119 Mo17-specific gene families (208 gene members).

In summary, a total of 9,058 B73 genes and 8,153 Mo17 genes had either large-effect mutations or large structural variations, as compared with their syntenic or best-hit Mo17 and B73 counterparts (Supplementary Table 13). Similarly, 8,278 and 9,738 PH207 genes had either large-effect mutations or large structural variations, as compared with the corresponding genes in B73 and Mo17, respectively (Supplementary Table 13). More than 20% of all predicted genes showed considerable protein sequence variations between any two inbred lines among B73, Mo17 and PH207, thus suggesting a potential functional complementation among these three representative maize inbred lines.

Notably, the proportion of genes with large-effect mutations and large structural variations within nonsyntenic genes was significantly higher than that in syntenic genes (chi-square test, \(P < 2 \times 10^{-16}\)). For example, the proportion of genes with large structural variations in the nonsyntenic gene group was with approximately ten times greater than that of the syntenic genes (Table 2).
and Supplementary Table 12). Interestingly, some of the nonsyntenic genes in the B73 genome had very high sequence homology with genes in Mo17 that were classified as syntenic genes. In fact, only 971 B73 nonsyntenic genes that had their best hits in the Mo17 genome were also identified as nonsyntenic genes. Among them, 479 genes were identified as mutual nonsyntenic best hits between B73 and Mo17. Our results showed that 1,529 B73 nonsyntenic genes with their best hits in Mo17 had syntenic homologs in B73. Similarly, 1,301 Mo17 nonsyntenic genes with their best hits in B73 had syntenic homologs in Mo17, thus indicating that a large proportion of these nonsyntenic genes are members of multiple gene families.

To examine the extent of gene amplification, we clustered all annotated genes in the B73, Mo17 and PH207 genomes. As a result, 3,589 (9.1%) B73, 5,044 (13.1%) Mo17 and 4,987 (13.3%) PH207 genes were identified as singleton genes (Supplementary Table 14). Most of the genes were amplified through either dispersed duplication (20,874 B73 genes; 19,468 Mo17 genes; and 20,082 PH207 genes) or whole-genome duplication and segmental duplication (20,874 B73 genes; 19,468 Mo17 genes; and 20,082 PH207 genes). The availability of high-quality assembled genomes for both B73 and Mo17 provides an unprecedented opportunity for extensive intraspecific genome comparison in maize. Direct comparison between the two maize genomes uncovered extensive SNP and indel variation, as well as a large extent of gene-order and structural variations. We demonstrated that more than 10% of B73 and Mo17 genes were mutually nonsyntenic, a value approximately two- to threefold higher than the proportion of nonsyntenic genes between R498 (indica) and Nipponbare (japonica) Asian rice (Supplementary Tables 13 and 19). Although some of the nonsyntenic genes have closely related homologs between these two maize lines, the positional changes of these genes may introduce different chromatin contexts affecting the expression of the genes themselves or their neighboring genes. The exact functions of these nonsyntenic genes are largely unknown, because they are underrepresented in classical genetics studies in maize. These nonsyntenic genes may play important roles in some lineage-specific functions, as demonstrated in a study on root development. Evaluating the contribution of these nonsyntenic genes to quantitative phenotypic variations of agronomic traits would be an interesting future pursuit.

In addition to the gene-order variations, there were several other types of intraspecific structural variations. Only 60% of the B73 and Mo17 genomes were able to be aligned as one-to-one blocks. Although the remaining 40% of the variable genome largely comprised repetitive elements, the B73 and Mo17 genomes each contained approximately 12 Mb of unique low-copy sequences, including 122 (72 in B73 and 50 in Mo17) high-stringency PAV genes. Furthermore, more than 20% of the annotated genes had large-effect mutations or large structural variations, which could potentially lead to protein sequence changes and potential functional

### Table 2 | Variations within genes between B73 and Mo17 genomes

| Variation type            | Syntenic genes | Nonsyntenic genes |
|---------------------------|----------------|-------------------|
|                           | B73 genes     | Mo17 genes        | B73 genes     | Mo17 genes     |
| Structurally conserved    | 28,122         | 28,186            | 1,534         | 1,216          |
| Without amino acid        | 12,167         | 12,674            | 326           | 306            |
| substitutions             |                |                   |               |                |
| No DNA variation in CDS   | 9,760          | 10,231            | 256           | 246            |
| region                    |                |                   |               |                |
| No DNA variation in CDS   | 6,870          | 7,344             | 169           | 169            |
| and intron region         |                |                   |               |                |
| No DNA variation in genic | 2,498          | 2,458             | 12            | 10             |
| region                    |                |                   |               |                |
| With amino acid changes   | 15,955         | 15,512            | 1,198         | 910            |
| With missense mutation in | 15,611         | 15,438            | 1,130         | 899            |
| CDS                       |                |                   |               |                |
| With 3n indel in CDS      | 5,941          | 5,632             | 186           | 221            |
| Genes with large effect   | 3,947          | 4,020             | 1,387         | 977            |
| mutations                 |                |                   |               |                |
| Start-codon mutation      | 240            | 374               | 175           | 109            |
| Stop-codon mutation       | 268            | 418               | 244           | 236            |
| Splice-donor mutation     | 170            | 124               | 73            | 37             |
| Splice-acceptor mutation  | 256            | 162               | 175           | 90             |
| With 3n ± 1 indel in CDS  | 2,044          | 1,983             | 547           | 384            |
| Premature stop codon       | 2,692          | 2,635             | 922           | 648            |
| Genes with large structural variations | 1,612 | 1,391 | 2,112 | 1,765 |
| At least one exon missing  | 1,025          | 811               | 1,725         | 1,508          |
| PAV genes                 | -              | -                 | 72            | 50             |
| Total                     | 33,681*        | 33,597*           | 5,105*        | 4,008*         |

*Only genes and their best hits in the counterpart genome anchored in ten pseudomolecules were included for the analysis. "Genic regions include 2 kb upstream and downstream of the gene body."
divergence between the two maize lines. Even after exclusion of potential redundancy resulting from multigene families, there were 320 B73-specific (and 170 Mo17-specific) gene families. In addition, there were 859 B73 genes and 770 Mo17 genes that showed specific expression in at least one of the tissues tested.

Several factors can contribute to the extensive intraspecific genome diversity observed. Transposable elements, such as helitrons, have been reported to be able to introduce gene movement or exon shuffling in the maize genome. The extensive genome diversity found suggests that the hybrids generated between two different maize lines may have a dramatically different complement of proteins or regulatory sequences, thus supporting the complementation hypothesis explaining the exceptional heterosis observed in maize nearly a century ago.

URLs: Falcon, https://github.com/PacificBiosciences/FALCON;/ Arrow, https://github.com/PacificBiosciences/GenomicConsensus;/ blasr, https://github.com/PacificBiosciences/blasr;/ Bwa, http://bio-bwa.sourceforge.net;/ Pilon, https://github.com/broadinstitute/pilon;/ IrysSolve, https://bionanogenomics.com/support/software-downloads/; GenBank, https://www.ncbi.nlm.nih.gov/genbank;/ RepeatModeler, http://www.repeatmasker.org/RepeatModeler;/ RepeatMasker, http://www.repeatmasker.org/RMDownload.html;/ Repbase, http://www.girinst.org/repbase;/ LRHarvest, https://genometools.org/index.html; LTRdigest, http://genometools.org/index.html; GyDB, http://gydb.org/; MUSCLE, https://www.drive5.com/muscle;/ EMBoss, http://emboss.sourceforge.net/; distmat, http://emboss.sourceforge.net/apps/release/6.6/emboss/apps/distmat.html;/ MAKER-P, http://www.yandell-lab.org/software/maker-p.html;/ Augustus, http://bioinf.uni-greifswald.de/augustus;/ FGENESH, http://www.softberry.com/berry.phtml;/ Munmer, http://mummer.sourceforge.net;/ Blastp, https://blast.ncbi.nlm.nih.gov/Blast.cgi;/ MCScanX, https://github.com/wyp1125/MCScanX;/ Phytozome, http://www.phytozome.net;/ OrthoMCL, http://orthomcl.org/orthomcl/;/ Synmap, https://genomevolution.org/coge/; last, http://last.cbrc.jp/; DAGchainer, http://dagchainer.sourceforge.net;/ Quota Aalign, https://github.com/tanghaibao/quota-alignment;/ PAML, http://abacus.gen.csc.uct.ac.za/software/paml.html.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41588-018-0182-0.

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**Author contributions**

J.L. designed the research. S.S., Y.Z. and Hainan Zhao performed genome assembly and genome annotation. S.S., Y.Z., J.P.S. and Hainan Zhao performed genome comparison. Haiming Zhao, J.C., W.S. and Y.C. prepared DNA/RNA samples and constructed the next-generation-sequencing library. J.L., S.S., Y.Z., J.C., Haiming Zhao, Hainan Zhao, W.S., M.Z., X.D., H.L. and X.M. participated in the analysis. Y.J., B.W., X.W., J.C.S., D.H.W. and C.L. were involved in gene annotation. J.C.G., F.L. and E.S.B. provided the anchored GBS tags. G.Y. was involved in the PacBio data generation. K.F., B.L., A.R. and P.S.S. participated in discussion on genome assembling and validation. J.L., S.S., Y.Z., J.C. and J.P.S. wrote the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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Methods

Plant material. The maize (Z. mays) inbred line Mo17 was selected for sequencing because of its important role in maize breeding and genetic research. The plants were grown in a greenhouse at 25 °C in dark conditions for 14 d, and the aerial parts of seedlings were harvested and frozen immediately in liquid nitrogen for extraction of genomic DNA. High-molecular-weight genomic DNA for library construction was extracted from isolated nuclei.

PacBio and Illumina library construction and sequencing. Libraries for SMRT PacBio genome sequencing were constructed as described previously. Approximately 20 µg of high-quality genomic DNA was sheared to an ~20-kb targeted size and assessed with an Agilent 2100 Bioanalyzer. Shearing of genomic DNA was followed by damage repair and end repair, blunt-end adaptor ligation and size selection with a Blue Pippin system (Sage Sciences). The final libraries were sequenced on the PacBio Sequel platform (Pacific Biosciences). Libraries for Illumina PCR-free paired-end genome sequencing were constructed according to the standard manufacturer's protocol (Illumina). Approximately 5 µg genomic DNA was fragmented, then size-selected (450 bp and 800 bp) through agarose gel electrophoresis. The ends of selected DNA fragments were blunted with an A-base overhang and ligated to sequencing adapters. After quality control, all the PCR-free libraries were sequenced on an Illumina platform with a paired-end sequencing strategy.

De novo assembly of PacBio SMRT reads. Approximately 24 million PacBio SMRT reads were used for contig assembly with Falcon. All reads were first pairwise compared to each other and compared with parameter ‘--length_cutoff 12000 --length_cutoff_pr 14000’. Preassembly and further error correction were then performed with parameters ‘--min_cov 4 --max_n_read 300’. The error rate used for overlapping detection was ‘--e 0.96’, and overlaps were filtered with the parameters ‘--bestn 10 --max_cov 60 --max_cov_2 --max_diff 60’. Overlapping graphs were constructed, and contigs were finally generated according to these graphs. All PacBio SMRT reads were then mapped back to the contigs with Blastz with the parameters ‘--bestn 10 --minMatch 12 --minSubreadLength 500 --minAlnLength 500 --minPctSimilarity 70 --minPctAccuracy 70’ --hitPolicy randombest --randomSeed 1. Arrow (see URLs) was used to correct the sequencing errors with default parameters according to the alignments. The Illumina PCR-free paired-end reads were mapped to the corrected contigs above with BWA mem with default parameters, and high-quality mapped reads (MAQ > 20) were further used to polish the assembly with Pilon with default parameters. This procedure resulted a total assembly length of 2.155 Gb with an N50 length of 1.48 Mb.

Construction of BioNano optical maps. High-molecular-weight DNA was isolated from the same tissue as described in the ‘Plant material’ section, digested by the single-stranded nicking endonuclease Nt.BspQl and then labeled with IrysPrep Labeling mix and Taq polymerase according to standard BioNano protocols. Labeled DNA was imaged automatically with the BioNano Irys system. BioNano raw BNX files were de novo assembled into genome maps with IrysSolve (see URLs). All single molecules were first sorted and autodenoised. Pairwise comparison was performed with RefAligner (see URLs) to identify all molecule overlaps, and consensus maps were constructed. All molecules were then mapped back to the consensus maps, and the maps were recursively refined and extended (five times). The final genome maps were 2.242 Gb in total, and the N50 of the maps was 1.411 Mb.

Hybrid assembly of PacBio contigs and BioNano optical maps. The BioNano IrysSolve (see URLs) module ‘HybridScaffold’ was used to perform the hybrid assembly between PacBio-assembled contigs and BioNano-assembled genome maps. To begin, we chose as follows: PacBio contigs were first converted into cma format. BioNano cnaps were then aligned to the contig cnaps with RefAligner, and this was followed by label rescaling. The rescaled BioNano cnaps were aligned again to the contig cnaps, and sequences were split at the conflict points. The ‘aggressive’ configuration was used, and scaffolds were built back to the alignment information between PacBio contigs and BioNano genome maps. There were 2,116 PacBio cnaps with a total length of 2.086 Gb linked by BioNano maps, which resulted in 348 scaffolds with an N50 of 10.51 Mb, with a total length of 2.12 Gb and ~40-Mb gaps introduced. The total length of the hybrid scaffolds and unscaffolded PacBio contigs was 2.18 Gb with an N50 of 10.42 Mb.

Assembly evaluation. We used published Mo17 BAC sequences to assess the quality of the hybrid assembly. Nine Mo17 BAC sequences (1,528 kb) downloaded from GenBank (see URLs) were mapped to the scaffolds with BLAT, and the alignment results were manually checked. All nine Mo17 BACs were covered by a single scaffold, with 100% coverage and 99.97% identity of the total BAC sequences, thus suggesting that the genome assembly was of high quality.

BWA mem was used to map the ~4.4 million maize GBS-tag sequences to the genome sequences to evaluate the assembly. The alignments were further filtered by mapping quality (MAQ = 60), and 36.68% were kept. Scaffolds with more than ten tags aligned were evaluated, and three scaffolds with tags aligned at disparate chromosome locations were split at the appropriate gap positions. After this correction, there were 2,560 scaffolds with an N50 length of 10.2 Mb, and the total genome length was 2.18 Gb. BUSCO2 was further used to evaluate the gene set assembled completed. Embryophytaodb20, which contained 1,440 single-copy orthologous genes was used as a searching dataset, and both Mo17 and B73 genomes were assessed.

Construction of pseudomolecules. The maize pangenome GBS tags were also used to anchor the assembled scaffolds onto Mo17 chromosomes. According to the mapping results above, scaffolds with more than ten tags aligned were further used to construct the pseudomolecules. The order and orientation of scaffolds were determined according to the physical positions of GBS tags. In total, 2.1-Gb scaffold sequences accounting for 96.4% of the Mo17 assembled genome were anchored onto the ten Mo17 chromosomes, which contained 97.95% of the annotated genes. We also aligned all GBS-tag sequences to the B73 genome and compared the alignments in these two genomes. The alignments showed high consistency between two genomes except for some pericentromere regions, owing to the lack of GBS tags.

Analysis of repetitive elements. We identified repetitive elements in the Mo17 genome through a combination of homolog-based and de novo approaches. RepeatModeler45 was first used to build TE consensus sequences as a de novo TE library on the basis of the Mo17 genome sequence. RepeatMasker50 was then used to discover and identify repeats in the Mo17 genome with the combined library of the de novo TEs of Mo17 and Repbase. Repetitive elements in the B73 and PH207 genomes were identified through the same method.

Transposon activity analysis. Full-length LTR retrotransposons were first identified in the assembled sequences of the B73, Mo17 and PH207 genomes withLTRharvest51 with the following parameters: ‘-longoutput -motif tCga -minlength 100 -maxlength 7000 -mindist 1000 0 -maxdist 20000 -similar 85 -motifs 1 -minid 5 -sdop 5 -olap 0.5 best overlaps best’. All predicted LTR retrotransposons were further annotated for protein domains with LTRdistiged with GtDB (see URLs) as a search database. Candidate with no typical protein domains (for example, GAG, INT, RT and RT) or a tandem-repeat content greater than 20% were filtered. This procedure resulted in a final set of 73,459, 74,160 and 50,402 high-confidence full-length LTR retrotransposons in the B73, Mo17 and PH207 genomes, respectively. To calculate the insertion age of each LTR retrotransposons, 5’ and 3’ LTRs of the same element were aligned with MUSCLE, and the distmat utility in the EMBOSS software package was used to calculate the accumulated divergence ‘K’ between 5’ and 3’ LTRs. Insertion times (T) of the LTR retrotransposons were calculated with the formula T = K/2 × r, where r is the TE-specific mutation rate of 1.3 × 10−8 per site per year.

RNA-seq data collection and generation. To aid in genome annotation and to perform transcriptome analysis, we generated RNA-seq data for five different tissues from B73 and Mo17: endosperm 12 d after pollination, 14-d seedlings, buds, ear and the silking stage. For the former two tissues, independent biological replicates were generated. All fresh tissues were frozen in liquid nitrogen and stored at −80 °C before processing. Total RNA of each sample was extracted with TRIzol according to the manufacturer’s instructions. RNA-seq libraries were prepared with the Illumina standard mRNA-seq library preparation kit and sequenced on the Illumina platform with paired-end sequencing strategy.

Gene annotation. MAKER-P version 3.1 (ref. 57) was used to annotate genes in the Mo17 genome, through a comprehensive strategy combining results obtained from protein-homology-based prediction, RNA-seq-based prediction and ab initio prediction. We used the same evidence that was used for the previous B73 gene annotation, with the addition of Mo17-specific RNA-seq datasets. Unannotated genes from Sorghum bicolor, Oryza sativa, Setaria italica, Brachypodium distachyon and Arabidopsis thaliana, downloaded from http://gramene.org/ release 48, were used for protein-homology-based prediction. 74,471 assembled transcripts from multiple Mo17 tissues, full-length transcripts from B73 Iso-seq3, and another set of 69,163 publicly available full-length CDNAIs from B73 deposited in GenBank, a total of 1,574,442 Trinity-assembled transcripts from 94 B73 RNA-seq experiments and 112,963 transcripts assembled from deep sequencing of a B73 seedling19 were collected and included as transcript evidence. Augustus74 and FGENESH (see URLs) were used for ab initio prediction of gene models in the TE-masked Mo17 genome. 44,747 genes (53,021 transcripts) were identified in the Mo17 genome and are referred to as the working gene set. This working set of gene annotations was expected to contain TEIs that were not masked before annotation or annotations with poor supporting evidence. We further filtered this working set according to AED scores generated in MAKER-P software, then confirmed splice sites and performed transposon screening. Finally, 38,620 high confidence genes remained and are referred to as the filter gene set.

Identification of SNPs and indels. We identified SNPs and insertion/deletion polymorphisms (indels, length <100 bp) between the B73 and Mo17 genomes with Mummer3 as follows: (i) The Mo17 pseudochromosome sequence was mapped...
to its corresponding B73 pseudochromosome with nucmer with the parameters

-mnurereference -g 1000 -c0 -140. (ii) The delta-filter was used to filter mapping noise and determine the one-to-one alignment blocks with parameters ‘-r’.

Alignments with aligned positions in one genome that were located more than 10 Mb away in another genome were further filtered. The aligned blocks between these two genomes were identified, and blank regions on the chromosomes that might be low-similarity regions or multiple aligned regions were filtered. (iii) Show-snps was used to obtain SNPs and small indels (<100 bp). B73-genome-based SNPs and indels were detected with the parameter ‘--clqTH’. Mo17- and Mo17-genome-based parameters were detected with the parameter ‘--clqTH’. SNPs and indels shared between the Mo17 and PH207 genomes, or the B73 and PH207 genomes, were processed with the same method used for SNPs shared between the B73 and Mo17 genomes. The genome distributions of SNPs and indels between the B73 and Mo17 genomes were also determined.

Identification of PAV sequences, PAV clusters and PAV genes. PAV sequences in the B73 and Mo17 genomes were identified through a sliding-window method. To identify B73 specific sequences, we divided the B73 genome into 500-bp overlapping windows with a step size of 100 bp and then aligned each 500-bp window with the Mo17 genome. Overlapping windows that could not be aligned were merged. Mo17- and PH207-specific sequences were identified through the same method. Most of these PAV sequences had a relatively short length (Supplementary Fig. 5), possibly as a result of our stringent calling criteria. We further merged PAV sequences that were within 100 kb of the physical coordinates to identify PAV clusters. If a merged region had more than 10% PAV sequences, we defined this region as a PAV cluster. We listed some large PAV clusters (>500 kb) in both the B73 and Mo17 genomes.

For the identification of B73-, Mo17- and PH207-specific genes, the CDSs of different transcripts were merged to represent a single gene, and genes with more than 75% of the CDS regions covered by PAV sequences were defined as PAV genes. We further aligned B73 resequencing reads from maize HapMap2 (ref. 36) to the Mo17 genome and Mo17 Illumina PCR-free reads to the B73 genome with BWA mem to exclude potential false positives. For the B73/Mo17-specific genes above, we filtered those with more than 50% CDS regions covered by resequencing reads to obtain the final PAV genes. Resequencing reads of 19 wild relatives, 23 landraces and 60 modern inbred lines from maize HapMap2 (ref. 36) projects were aligned to the B73, Mo17 and PH207 genomes with BWA mem. The mapping depth across the PAV gene regions was calculated with samtools (1 nt) that might produce initiation codons, termination codons, premature termination, splicing donor-site or splicing-acceptor-site mutations, and ORF frame-shifts were classified as genes with large-effect mutations. The remaining genes not identified as PAV genes were classified as genes with large structural variation.

For the synteny-based method, the best hits located in the synteny regions were used to assess gene-structure variation. Structurally conserved genes and genes with large-effect duplications were defined and aligned to the defined criteria above. The remaining genes with more than 75% CDS missed in the synteny regions were classed as genes with syntenic information not established or were otherwise classified as genes with large structural variation. Notably, only genes and their best hits in the counterpart genome anchored in ten pseudomolecules were included in the analysis. The comparisons of PH207 genes to the B73 and Mo17 genomes were performed in the same way.

Identification of duplicated genes and gene families. To identify gene duplications, BLASTP was used to calculate pairwise similarities ($e$-value < 1 x 10$^{-5}$), and MCscanX with default parameters was then used for classification.

To identify gene families, we merged annotated genes from Mo17, B73 and three other grasses from the Phytome database (see URLs), including S. bicolor (33,032 genes), O. sativa (39,049 genes), B. distachyon (31,694 genes) and A. italiana (27,416 genes). The longest proteins for each gene were aligned to one another. BLASTP was used to calculate pairwise similarities ($e$-value < 1 x 10$^{-5}$), and OrthoMCL was used to identify gene families with an inflation value of 2 and percent-match cutoff of 50.

Comparative genomic analysis among B73, Mo17, PH207 and sorghum. To perform the comparative genomic analysis, we used the Synmap pipeline (see URLs). In brief, we used lastZ to blast the CDS sequence, then detected syntenic blocks with DAgChainer with options -D 20 -A 5. Quota Align was further used to merge adjacent syntenic blocks. The syntenic depth was set to 2:1 for maize and sorghum in one genome and 1:1 for B73 and Mo17, B73 and PH207, and Mo17 and PH207 comparisons, and the overlapped distance was set to 40 to permit overlapped syntenic regions. Fractionation bias was applied to determine subgenome organization in maize compared with sorghum. The CodeML utility in the PAML software package was used to calculate the Ka and Ks rates between orthologous genes. The time of divergence from the common ancestors of maize (~2.1 Ma) was inferred on the basis of the Ks of 0.025, because maize and sorghum shared a common ancestor with ~11.9 Ma (Ks ~0.139).

Transcriptome comparison between B73 and Mo17. The RNA-seq data for the bract, root, stem, endodermis and pericarp tissues from the B73 and Mo17 lines were used to perform transcriptome comparison between B73 and Mo17. The RNA-seq data were aligned to both the B73 and Mo17 genomes with Hisat2 (ref. 39). All aligned reads were used to calculate the fragments per kilobase per million (FPKM) values with Cufflinks. Genes with FPKM value greater than 1 for tissues of B73 (Mo17) and lower than 0.1 in corresponding tissues of Mo17 (B73) were deemed B73 (Mo17) specifically expressed genes.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Code availability. Custom codes used in this study are currently hosted in a GitHub repository at https://github.com/caudal/Mo17_genomeAssembly.

Data availability. The genome assembly and gene annotation have been deposited in the NCBI database under BioProject number PRJNA358298 and BioSample number SAMN0619745. The GenBank accession number of the above data is NCVC00000000. Raw PacBio SMRT reads, Illumina data and RNA-seq data have been deposited in the NCBI SRA database under access number SRP111315.

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| ☑   | Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated |
| ☑   | Clearly defined error bars |
| ☑   | State explicitly what error bars represent (e.g. SD, SE, CI) |

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection  Pacbio sequel platform produce the SMRT long reads; Bionano irys platform produce the labeled DNA molecules; Illumina Hiseq 2000 platform produce PCR-free pair-end reads and RNA-seq reads.

Data analysis  Falcon v0.3.0; Arrow v2.1.0; blasr; bwa v0.7.12; Pilon v1.22; RepeatModeler v1.0.10; RepeatMasker v4.0.7; LTRharvest v4.4.7; LTRdigest v4.4.7; EMBoss; MAKER-P, v3.1; Augustus v3.1; FGENESH; Blast+ v2.2.28+; MCscanX; OrthoMCL v5; Synmap; last v926; DAGchainer r020208; Quota Align; PAML v4.9; All custom codes used in this study will be made available according to the request.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The genome assembly and gene annotation can be achieved from NCBI with BioProject number PRJNA358298 and BioSample number SAMN06169745. The GenBank accession number of the data above is NCVQ00000000. Raw PacBio SMRT reads, Illumina data, and RNA-seq can be downloaded from NCBI SRA database with accession number SRP111315.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [x] Life sciences
- [ ] Behavioural & social sciences
- [ ] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
No sample size calculation was performed, as we choose only one maize inbred lines to sequence and assemble.

Data exclusions
NA

Replication
NA

Randomization
NA

Blinding
NA

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a
- [x] Unique biological materials
- [x] Antibodies
- [x] Eukaryotic cell lines
- [x] Palaeontology
- [x] Animals and other organisms
- [x] Human research participants

Methods

n/a
- [x] ChIP-seq
- [x] Flow cytometry
- [x] MRI-based neuroimaging