European maize genomes highlight intraspecies variation in repeat and gene content

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The diversity of maize (Zea mays) is the backbone of modern heterotic patterns and hybrid breeding. Historically, US farmers exploited this variability to establish today's highly productive Corn Belt inbred lines from blends of dent and flint germplasm pools. Here, we report de novo genome sequences of four European flint lines assembled to pseudomolecules with scaffold N50 ranging from 6.1 to 10.4 Mb. Comparative analyses with two US Corn Belt lines explain the pronounced differences between both germplasms. While overall syntenic order and consolidated gene annotations reveal only moderate pangenomic differences, whole-genome alignments delineating the core and dispensable genome, and the analysis of heterochromatinic knobs and orthologous long terminal repeat retrotransposons unveil the dynamics of the maize genome. The high-quality genome sequences of the flint pool complement the maize pangenome and provide an important tool to study maize improvement at a genome scale and to enhance modern hybrid breeding.

Since its domestication ~10,000 years ago by Native Americans, maize (Zea mays ssp. mays) has become one of the most important sources for human nutrition and animal feeding. Extensive variation in landraces and breeding germplasm such as dent or flint corns underpins the enormous phenotypic and genetic diversity of maize⁴,⁵. Today, US hybrids produced from inbred lines of different heterotic groups; for example, stiff stalk and nonstiff stalk are highly productive and agriculturally important worldwide. These US Corn Belt dents resulted from crosses in the nineteenth century between Southern dent lines introduced to the United States from Mexico and Northern flints (NF) which were the predominant germplasm in the pre-Columbian era grown by the Native Americans⁶-⁸. Historical records and genetic and molecular data strongly indicate that NFs originated from Native American populations of Southwestern America and the Great Plains⁹-¹⁰. Improvement by early farmers and adaptation to cooler climate and different photoperiods extended NF growth as far north as Southern Canada. After the colonization of the New World, maize was spread to Europe both from the Caribbean islands and Northeast United States¹. The early maturing and cold-tolerant flints were key to a successful maize cultivation in temperate regions of Europe. Consequently, NF germplasm still makes a major contribution to modern European maize breeding material while Corn Belt dent genomes contain on average one-quarter from their blending with NFs²⁴.

The complete genome sequence of the dent line B73 and its continuous updates provide a high-quality reference for maize breeding, genetic and genomic research³⁴,²⁶,²⁷. However, very high diversity at both the sequence and genic level has been reported between maize inbred lines for targeted regions and large-scale comparisons³⁴,⁴¹-⁴₄. Hence, the B73 reference sequence captures only a portion of the maize pangenome. To overcome these limitations, a draft genome of the lodent line PH207 (ref. ¹) and—more recently—reference sequences of SK, Mo17 and W22, a tropical and two US dent lines, have been released³⁴,⁴₅,⁴₆. Worldwide, many hybrid breeding programs focus on dent germplasm, whereas breeding programs in cooler regions of Central Europe exploit heterotic effects between dent and flint lines. Several studies have shown a clear differentiation of the North American and Northern European flint germplasm from the rest of the world²⁴. While reference-quality sequences exist for several dent inbred lines, the flint pool is still underexploited. To date, only one fragmented draft assembly with an N50 ~13.9 kilobases (kb) of one flint line, F2, is available covering ~65% of the estimated genome size²⁴. To better understand the extent and quality of genomic differences between the flint and dent germplasm pools we generated de novo high-quality sequences for four flint inbred lines representing important ancestors of maize hybrid breeding in Central Europe²⁴ and contrasted in this study their gene and repeat contents to two US Corn Belt dents.

Results

Whole-genome sequencing. To access the genomic landscape of European flint maize, we assembled four flint lines to reference genome quality. Three lines are important founders of European breeding programs and represent distinct flint germplasm sources such as populations Lacaune (F7, Southern France), Lizargarate (EP1, Northern Spain) and Gelber Badischer Landmais (DK105, Southern Germany). The fourth sample, PE0075, is a doubled-haploid line derived from the Petkuser Ferdinand Rot population, a landrace from Northern Germany. We generated Illumina paired-end and mate-pair sequences equivalent to ~220–320x coverage (Supplementary Table 1) and assembled the reads to

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scaffolds and pseudomolecules using the DeNovoMagic pipeline\(^1\). Total assembly sizes of the pseudochromosomes range from 2.14 to 2.32 megabases (Mb) and amount to 92.9–100.5% of the genome sizes estimated by K-mer analysis (Table 1 and Supplementary Fig. 1). Most (94.3–97.3%) contig and scaffold sequences were integrated into ten pseudochromosomes (Table 1, Supplementary Table 2 and Extended Data Fig. 1a). A genetic map generated from an F\(_1\) mapping population of an EP1 × PH207 cross demonstrated a high consensus between genetic and physical map corroborating quality and contiguity of the maize assemblies (Extended Data Fig. 2). Sequence accuracy was high with less than one erroneous base per 100 kb (Supplementary Table 3) and remapping genomic reads using SQUAT\(^2\) reported 2% poorly mapped reads. Complete BUSCO\(^4\) genes totaled >95%, strongly supporting high coverage of the gene space while only ~1.5% and 0.8–2.4% of the BUSCO Liliopsidae set were absent or fragmented in each of the four assemblies on average, respectively (Supplementary Table 2). In addition, the long terminal repeat (LTR) assembly index\(^5\), a measure for the correct reconstruction of the repetitive transposon space, supports the reference quality of our four flint assemblies (Supplementary Table 4).

**Pan-gene variation among flint and dent is moderate.** To assess genomic presence–absence variations (PAVs) in the six maize lines we used a three-layer gene prediction pipeline. For F7 and EP1 we predicted protein-coding genes as consensus models using protein homologies of known monocotyledonous protein sequences and a broad spectrum of transcriptome evidences from F7 and EP1 RNA-seq data (Supplementary Table 5). Subsequently, cross-mapping of the EP1 and F7 models as well as annotations of B73 (v.4) and PH207 (v.1.1) complemented the genetic sets of all six maize lines including PE0075 and DK105. In total, we identified ~46,200–48,000 consolitated gene models per line (Table 1). Cross-mapping and consolidation substantially improved the number of pairwise reciprocal best blast orthologs (Fig. 1a and Supplementary Table 6) as well as the completeness of orthologous clusters (Fig. 1b and Supplementary Fig. 2).

Overall, 43,700–45,900 genes (94.7–98% of all genes per line) have an orthologous counterpart or reciprocal best blast match in at least one of the other lines (Fig. 1b). Comparison of the original and consolidated annotations of B73 and PH207 suggests that part of this gene complement displays functional signatures such as homology to angiosperm proteins or expression in the respective line (Fig. 1a and Supplementary Fig. 3). The cross-mapped annotation significantly improved the completeness of syntegol clusters with 34,352 clusters that have syntegols for all six lines, and 94.6% of these contain exactly one gene copy per line while 1,861 (5.4%) contain additional co-orthologous members (Fig. 1b). Genic PAVs strongly support flint and dent as distinctive germplasm (Extended Data Fig. 3). Overall, core genes present in all six maize lines show high expression levels (mean and median 25.8 and 5.9 transcripts per million, TPM) while PAV genes show significantly lower expression levels (median 0.02–0.06 TPM) and have lower homology support in a comparison to 27 angiosperms (Fig. 1c,d). Note that genes found in only one line in general show more pronounced support for protein-coding sequences compared to genes of cluster sizes 2–4. Genes with no corresponding maize ortholog (cluster size 1) are enriched for tandem duplications (Fisher exact test with \(P\) values between \(<1 \times 10^{-8}\) and \(1 \times 10^{-70}\)). The very low median expression of syntenic dispensable and singleton genes suggests no, only minor or highly specialized line-specific functions for most of these genes.

Consistent with the genome analysis of the maize inbred line Mo17 (ref. 17), our cross-consolidation revealed a sizeable number of predicted genes with large effect mutations. Between 2,487 (B73) and up to 4,264 (DK105) gene models had no contiguous ORF due to internal stop codons or frameshifts. Genes with disrupted ORFs had highly similar expression levels (Pearson correlation 0.93 < \(r< 0.95\)) to their syntenic partner with contiguous reading frame (Extended Data Fig. 4). Close inspection revealed a series of reasons including pseudogenization, missing splice variants or skipped exons in the respective line.

### Dynamics of the full-length retrotransposon landscape

Approximately 80% of the maize genome is constituted by repeats of different types. In addition to the six lines above, the recently published genomes Mo17 and W22 were analyzed for their repeat content\(^18,19\). No pronounced differences in transposable element (TE) composition among the eight lines analyzed was detected (Extended Data Fig. 1 and Supplementary Table 7). We detected almost 15,000 high-quality full-length LTRs (fl-LTRs) per line, matching the expected genome size to fl-LTR ratio\(^2\) (Supplementary Table 8a). The lower number observed for PH207 (6,838 fl-LTRs) can be attributed the overall lower quality assembly compared to the other five lines. Correspondingly, the PH207 assembly was excluded from evaluation depending on the analysis criteria. For the remaining five lines, the number and age distribution of fl-LTRs confirm the high quality of the respective assemblies (Extended Data Fig. 5).

To quantify transposon dynamics between the different maize lines we identified still-shared syntonic fl-LTRs by clustering TE junctions with high stringency. Only 3% of all fl-LTR locations were found to be shared between six lines. While the percentage of shared elements among the different lines shows a marked decrease, the
Heterochromatic knob islands in the maize genome. Knob regions are heterochromatic regions in the genome that have been demonstrated to affect local recombination\textsuperscript{26,27}. They belong to the group of satellites tandem repeats which are well represented in our flint assemblies (Extended Data Fig. 6a, Supplementary Fig. 4 and Supplementary Table 8b). Maize knobs are composed of 180 bp and closely related 202-bp tandemly repeated sequence units (Extended Data Fig. 6c). Variations in position and extent of knob regions in maize have been documented\textsuperscript{26,28}. Intensity and position of knob regions detected by FISH for the different karyotypes clearly separate dent from flint lines (Fig. 3a and Extended Data Fig. 6b). In particular, extensive knob regions detected on chromosomes 7 and 8 of the dent lines are absent in flint lines. For other positions on the genome, a clear flint/dent separation among lines cannot be observed. However, the observed variations reveal the pervasive dynamics of knob regions in the genome. FISH requires a minimum number of adjacent tandem units for their detection. High-quality genome assemblies resolving a larger proportion of the repetitive space allow analyzing the so-far-hidden, cryptic, knob sites which we annotated by homology to the 180 and 202 bp monomers. Besides the large knob regions detected by FISH, numerous additional positions with fewer tandem repeat units are found in the sequence assembly on all chromosomes (Fig. 3b and Extended Data Fig. 7). A strikingly pronounced syntenic conservation of minor knob sites common to all analyzed flint and dent lines was observed. Given the observations of rapid diversification based on repeat units detailed above, this might indicate numerous potential (shadowed) knob regions that can rapidly expand or shrink by, for example, illegitimate recombination and can cause recombinational isolation of the affected regions.

In fission yeast, knob structures have been shown to implicate significant downregulation of surrounding genes\textsuperscript{39}. To check for similar effects in maize we analyzed the distance of genes to the nearest knob signature in the assembly and related it to expression data\textsuperscript{39} generated for the lines B73, EP1 and F7. Even more pronounced than observed for other types of repeat elements, we find the close...
surrounding of knob repeat units to be devoid of genes (Fig. 3c). For genes surrounding knob regions, we observe a trend for lower expression values (Extended Data Fig. 7d).

**Whole-genome alignment.** Numerous large structural variations (SV) in maize have been reported\(^{1,11,12,14}\). To gain insight into the extent of SVs in the maize flint versus dent genomes we generated pairwise whole-genome alignments (WGAs) for the six lines and postprocessed the resulting alignments to single alignment blocks (SAB) that represent the highest scoring one-to-one relationships between each genome pair. To approximate overall contiguity between two genomes, SABs were further concatenated to merged alignment blocks (MABs) if they followed a strict and unambiguous order in both genome sequences (Supplementary Fig. 5). On average, ~50% of the genome sequence aligned in each of the pairwise comparisons by SAB scoring, while WGAs (MABs) associated 80–90% (1.7–2 Gb) of the genome sequences (Supplementary Tables 9 and 10). Mean sizes of SABs and MABs were ~10 kb and ~100 kb, respectively (Supplementary Fig. 6). Evaluation of pairwise WGA regions strongly supported orthology of segments in parallel orientations (Supplementary Fig. 7) while an assessment of most rearrangements (inversions and transpositions) was inconclusive due to insufficient or ambiguous information at the breakpoints.

To delineate regions that do not align with any other of the five lines (unaligned), align solely with flint or dent maize lines (group-specific) or are aligned in all pairwise alignments (core), we combined pairwise WGAs of each line and projected them onto its genome sequence (Supplementary Fig. 8). We classified a genomic region as core type if it aligned to four out of the five possible lines due to insufficient or ambiguous information at the breakpoints.
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(Fig. 4a and Extended Data Fig. 8). Notably, for all six lines highest core densities located at regions adjacent to the centromere and correspond to above-average SAB sizes and below-average recombination rates (Fig. 4b). These findings are reminiscent of observations in the Triticeae gene space22 mirroring the recombinogenic properties of centromeric and telomeric regions.

Genetic mosaicism in US dents and European flints. The core genomic regions served as positional anchors to generate multiple sequence alignments. One-to-one unambiguous alignments for the six lines total 287 Mb (~15%) of each genome and comprise $6.25 \times 10^6$ orthologous SNPs with positional information for each of the six genomic coordinate systems. The detected SNPs showed an excellent agreement of 99.3% (4,827 mismatches out of 653,398 total scored calls) to a published set of SNP calls using the Affymetrix Axiom Maize Genotyping Array with 600,000 variants31. On the basis of sequences of identical SNP pairs, we determined significant haplotypes between all 15 line combinations (Fig. 4c). Mean and total genomic spans covered by these pairwise near-identical haplotypes range from 40 to 68 kb and 394 to 664 Mb, respectively. The proportion of one-fifth to one-third shared genomic regions between flint and dent lines is in close agreement with previous studies of NF content in stiff stalk and nonstiff stalk dents2,6. Number, mean and total sizes were highly consistent with their phylogenetic relationship.
region covered, the top five/six haplotypes encode combinatorial groupings with five out of six lines being identical. The haplotype distinguishing between the studied dent and European flint lines ranked only as the sixth (by size) or seventh (by SNP number) most frequent (Fig. 4d and Supplementary Table 11). The distinct higher order haplotypes distribute evenly along the ten chromosomes but also comprised several striking clusters. Although not the most prominent identical-by-descent type, three regions were enriched for the haplotype distinctive between flint and dent lines: two at the distal sites of chromosomes 4 and 7 and a large portion spanning ~12 Mb in the proximal part of chromosome 8. The latter strongly coincides with group-specific WGAs on chromosome 8 (Extended Data Fig. 8) and encloses the major flowering time quantitative trait locus vgt1 in maize32,33.

Haplotype-informed differential gene expression analysis. To explore putative functional consequences of the haplotype differentiating our dent and European flint lines, we analyzed its effect on the maize transcriptome. From the maize association panel we selected a subset of 40 out of 282 lines, which were highly similar to either B73 or F7/EPI within regions defined by this haplotype (Supplementary Fig. 9)34,35. We analyzed genome-wide expression levels between the two contrasting groups underlying three different genome references (B73, EPI and F7) and using expression data from seven different conditions and tissues36. In total, 4,761 genes orthologous between B73, EPI and F7 (out of 35,389 triplets) were differentially expressed (DEGs) in all three references and in at least one of the tissues and conditions (Fig. 5).

Comparing identified DEGs with curated maize genes revealed a striking pattern for genes involved in the establishment of the kernel texture. We identified a plethora of genes involved in starch metabolism: sugar transporters including several proteins of the SWEET family, hex4 and hex7, sugar transporter stp1, the rate-limiting gene for starch biosynthesis (shrunken2), starch synthase1 (s1), starch phosphorylase, the α(1→6)-glucosylhydrolase sugary (sug) and starch branching enzyme shel catalyzing the formation of α(1→6)-glycosidic bonds in amylopectin (Fig. 5c). We also detected a number of genes that establish and regulate the prolamin content and structure of the maize endosperm (Fig. 5b) including opaque endosperm o7 and o10, empty pericarp e16, floury endosperm f13 and naked endosperm nkd137–40.

Discussion

The comparative analysis of the gene and repeat content together with the analysis of WGAs and SNPs confirms the European flints as highly differentiated germplasm of cultivated maize at a whole-genome scale. For all these analyses, the four flint and the two dent lines form two distinct groups, with each line sharing larger numbers of syntenic genes, repeats, aligned regions and haplotypes with members of the same group than with any member of the other group. Nevertheless, we also observed significant overall conservation of chromosome structure and gene content. Aligned blocks ordered by consecutive syntenic order span ~85% of the entire genome in all lines. Cross-consolidation of gene models revealed a moderate number of nonsyntenic genes, suggesting that genomic PAVs are less pronounced in maize than previously reported. Consistently, significant lower support by expression and homology indicates that less conserved orthologous clusters are likely enriched for nonfunctional and poor gene models. Nevertheless, the latter set also contains many genes expressed at moderate to high levels, which might contribute to line-specific adaptations and are of interest for maize improvement and breeding. Additionally, some of these genes may have highly confined expression patterns or conditions that were missed in our transcriptome analysis. The cross-consolidation approach also had several limitations including the potential transfer of poor gene models and the identification...
of genes that contain large effect mutations and disrupted ORFs. Inspection of the latter set revealed several causes including likely true pseudogenizations or truncations, probable differences in the exon–intron structure between the applied informant and target gene model as well as mapping errors.

In contrast to the overall syntenic conservation, a large amount of unaligned, nonorthologous sequences for WGAs and the analysis of the repeat space demonstrated enormous diversity and dynamics of the maize genome. Only 2% of shared orthologous fl-LTRs are conserved in eight lines. Consistent with previous reports, large FISH visible knobs display substantial variation in position among the maize lines studied. Remarkably, both shared sets of orthologous LTRs and larger knob positions perfectly reproduced the relationship of the six lines based on coding sequences, indicating smooth transitions between these features during breeding and admixture. In contrast to previous studies reporting low knob numbers in NF or even knob-less NF lines, the European flints contained similar knob numbers to the US dents. It is unclear whether this is a characteristic of the NF ancestral lines migrated to Europe, reflects the breeding history in European flints or represents a technical advantage given the progress in methodology to detect weaker signals.

Consistent with fiber-FISH studies, we also detected numerous locations of the 180-bp knob repeat unit throughout the chromosome assemblies. The potential contribution of these widespread knob repeats on gene density, expression levels and rare deleterious alleles requires future studies and adds further complexity to known effects of large-scale knobs in maize on recombination rates, meiotic drive and phenotypic traits such as flowering time.

WGAs enabled us to identify long runs of pairwise and higher order combinations of genomic regions with identical haplotypes. Size and distribution of these haplotype blocks reflect complexity of historic recombination, intercrossing and breeding history over the last centuries. Similar to the gene and repeat comparisons, the SNP runs highlight the separation of flints and dents of this study but also illustrate and delineate candidate regions of common ancestry in the US Corn Belt and European flint lines. We identified a set of regions that differentiated the studied Corn Belt dents from the European germplasm. Intriguingly, genes...
differentially expressed between two groups of maize varieties that have been selected by their genotypic similarity to these regions comprised a nearly complete starch biosynthesis pathway and several genes involved in the establishment and organization of the endosperm texture. Hence, besides the detection of selective sweeps and other genomic signatures, it might be equally informative to survey trans-effects of such genomic regions.

Given the high dynamics and tessellation of the maize genome, we estimate that many high-quality genome sequences will be required for improving breeding efficacy and understanding maize biology. The four European flint lines of this study close an important gap in our knowledge and complement the maize pan-genome.

Online content
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References
1. Doebley, J. F., Goodman, O. M. & Stuber, C. W. Exceptional genetic divergence of northern flint corn. Am. J. Bot. 73, 64–69 (1986).
2. Liu, K. et al. Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. Genetics 165, 2117–2128 (2003).
3. Chia, J. M. et al. Maize HapMap2 identifies extant variation from a genome in flux. Nat. Genet. 44, 803–807 (2012).
4. Davick, D. N. The contribution of breeding to yield advances in maize in flux. Nat. Genet. 44, 803–807 (2012).
5. Anderson, E. & Brown, W. L. The History of the Common Maize Varieties of the United States Corn Belt (Univ. of California Press, 1952).
6. Doebley, J. et al. The origin of cornbelt maize: the isozyme evidence. Econ. Bot. 42, 120–131 (1988).
7. van Heerwaarden, J., Hufford, M. B. & Ross-Ibarra, J. Historical genomics of North American maize. Proc. Natl Acad. Sci. USA 109, 12420–12425 (2012).
8. Tenlaufon, M. I. & Charcosset, A. A European perspective on maize history. C. R. Biol. 334, 221–228 (2011).
9. Jiao, Y. et al. Improved maize reference genome with single-molecule technologies. Nature 546, 524 (2017).
10. Schnable, P. S. et al. The B73 maize genome: complexity, diversity, and dynamics. Science 326, 1112–1115 (2009).
11. Lai, J. et al. Gene loss and movement in the maize genome. Genome Res. 14, 1924–1931 (2004).
12. Springer, N. M. et al. Maize inbreds exhibit high levels of copy number variation (CNV) and presence/absence variation (PAV) in genome content. PLoS Genet. 5, e1000734 (2009).
13. Swanson-Wagner, R. A. et al. Pervasive gene content variation and copy number variation in maize and its undomesticated progenitor. Genome Res. 20, 1689–1699 (2010).
14. Wang, Q. & Dooner, H. K. Remarkable variation in maize genome structure inferred from haplotype diversity at the b locus. Proc. Natl Acad. Sci. USA 103, 17644–17649 (2006).
15. Hirsch, C. N. et al. Draft assembly of elite inbred line PH207 provides insights into genomic and transcriptomic diversity in maize. Plant Cell 28, 2700–2714 (2016).
16. Springer, N. M. et al. The maize W22 genome provides a foundation for functional genomics and transposon biology. Nat. Genet. 50, 1282–1288 (2018).
17. Sun, S. et al. Extensive intraspecific gene order and gene structural variations between Mo17 and other maize genomes. Nat. Genet. 50, 1289–1295 (2018).
18. Yang, N. et al. Genome assembly of a tropical maize inbred line provides insights into structural variation and crop improvement. Nat. Genet. 51, 1052–1059 (2019).
19. Unterseer, S. et al. A comprehensive study of the genomic differentiation between temperate Dent and Flint maize. Genome Biol. 17, 137 (2016).
20. Darraaq, A. et al. Sequence analysis of European maize inbred line F2 provides new insights into molecular and chromosomal characteristics of presence/absence variants. BMC Genomics 19, 119 (2018).
21. Messmer, M. M. et al. RFLP analyses of early-maturing European maize germ plasm: I. Genetic diversity among flint and dent inbreds. Theor. Appl. Genet. 83, 1003–1012 (1992).
22. International Wheat Genome Sequencing Consortium et al. Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science 361, 7191 (2018).
23. Yang, L. A. et al. SQUAT: a Sequencing Quality Assessment Tool for data quality assessments of genome assemblies. BMC Genomics 19, 238 (2018).
24. Simão, F. A. et al. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31, 3210–3212 (2015).
25. Ou, S., Chen, J. & Jiang, N. Assessing genome assembly quality using the LTR Assembly Index (LAI). Nucleic Acids Res. 46, e126 (2018).
26. Ghaffari, R. et al. Maize chromosomal knobs are located in gene-dense areas and suppress local recombination. Chromosoma 122, 67–75 (2013).
27. Stack, S. M. et al. Meiotic crossing over in maize knob heterochromatin. Genetics 205, 1101–1112 (2017).
28. Ananvier, E. V., Phillips, R. L. & Rines, H. W. Complex structure of knobs and centromeric regions in maize chromosomes. Tidiot. Genet. 34, 11–15 (2000).
29. Matsuda, A. et al. Highly condensed chromatin are formed adjacent to subtelomeric and decondensed silent chromatin in fission yeast. Nat. Commun. 6, 7753 (2015).
30. Kremling, K. A. et al. Dysregulation of expression correlates with rare-allele burden and fitness loss in maize. Nature 555, 520 (2018).
31. Unterseer, S. et al. A powerful tool for genome analysis in maize: development and evaluation of the high density 600 k SNP genotyping array. BMC Genomics 15, 823 (2014).
32. Salvi, S. et al. Conserved noncoding genomic sequences associated with a flowering-time quantitative trait locus in maize. Proc. Natl Acad. Sci. USA 104, 11376–11381 (2007).
33. Ducrocq, S. et al. Key impact of Vg1 on flowering time adaptation in maize: evidence from association mapping and ecogeographical information. Genetics 178, 2433–2437 (2008).
34. Bukowski, R. et al. Construction of the third-generation Zea mays haplotype genome. Gigasize 7, 1–12 (2018).
35. Cook, J. P. et al. Genetic architecture of maize kernel composition in the nested association mapping and inbred association panels. Plant Physiol. 158, 824–834 (2012).
36. James, M. G., Robertson, D. S. & Myers, A. M. Characterization of the maize gene sugary1, a determinant of starch composition in kernels. Plant Cell 7, 417–429 (1995).
37. Yang, J., Ji, C. & Wu, Y. Divergent transactivation of maize storage protein zein genes by the transcription factors Opaque2 and OHPs. Genetics 204, 581–591 (2016).
38. Yao, D. et al. Maize opaque10 encodes a cerealspecific protein that is essential for the proper distribution of zeins in endosperm protein bodies. PLoS Genet. 12, e1006270 (2016).
39. Yi, G. et al. The naked endosperm genes encode duplicate INDETERMINE domain transcription factors required for maize endosperm cell patterning and differentiation. Plant Physiol. 167, 443–456 (2015).
40. Zhang, S., Zhan, J. & Yang, Z. Maize opaque mutants are no longer so opaque. Plant Reprod. 31, 319–326 (2018).
41. Brown, W. L. Numbers and distribution of chromosome knobs in United States maize. Genetics 34, 524–536 (1949).
42. Adawy, S. S., Supar, R. M. & Jiang, F. Fluorescence in situ hybridization analysis reveals multiple loci of knob-associated DNA elements in one knob and knobless maize lines. J. Histochem. Cytochem. 52, 1113–1116 (2004).
43. Jian, Y. et al. Maize (Zea mays L.) genome size indicated by 180-bp knob repeats of maize. Theor. Appl. Genet. 109, 11376–11381 (2007).
44. Kanzjazz, I. B. et al. Intragenomic conflict between the two major knob repeats of maize. Genetics 194, 81–89 (2013).
45. Huffman, M. B. et al. Comparative population genomics of maize domestication and improvement. Nat. Genet. 44, 808 (2012).
46. Lai, J. et al. Genome-wide patterns of genetic variation among elite maize inbred lines. Nat. Genet. 42, 1027 (2010).
47. Reif, J. C. et al. Trends in genetic diversity among European maize cultivars and their parental components during the past 50 years. Theor. Appl. Genet. 111, 838–845 (2005).

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Methods

Plant material and genome assembly. The four flint inbred lines were chosen to represent landraces from different European ancestry. While PE0075 (a doubled-haploid line derived from the landrace Petkuser Ferdinand Rot) and DK105 (derived from Gelerl Badischer Landmais) can be classified as representatives of the NE, EP1 and F7 represent Pyrenean–Galician ancestry and were derived from populations Lizaragarte and Laucane, respectively. Contigs, scaffolds and pseudochromosomes were assembled de novo using the DeNovoMAGIC 2.0 technology for lines EP1 and F7, and DeNovoMAGIC 3.0 for lines DK105 and PE0075 (Emsong R Technologies 2009), respectively (Supplementary Table 2) using the B73 (v4) reference genome to assist anchoring and orienting scaffolds and contigs to pseudochromosomes. The application of this toolset has been described previously for several plant genome assemblies. Briefly, bulked leaf tissue from 15–24 seedlings was harvested, immediately ground in liquid nitrogen and genomic DNA was then isolated using a modified CTAB extraction protocol. Genomic DNA sequencing of paired-end and mate-pair libraries was performed as specified in Supplementary Table 1. Remappings of the paired-end and mate-pair libraries were used to estimate gap sizes in the assemblies (Supplementary Fig. 10).

A cross of parental lines PH207 × EP1 was used to establish a high-density genetic map from F1 mapping populations. Parents and 192 F2 plants were analyzed using the Affymetrix Axiom Maize Genotyping Array and data were processed as described in ref. 31, which resulted in 174,616 markers. The markers were clustered into groups showing identical segregation patterns using POPSEQ. A first genetic map was calculated with 9,404 binmap markers with the R package ASMap v.1.0-2 (ref. 52) using the function mstmap with the following parameters: pop.type="RIL2", dist.fun="kossamb", objective.fun="COUNT", p.value=1e-22, noMap.dist=15, noMap.size=2, miss.thresh=0.00. Linkage groups were assigned to the ten maize chromosomes on the basis of previously mapped markers. Unlinked small groups with only a few markers were discarded. Ten F2 plants exhibited very high numbers of crossovers and were excluded from further analyses. In multiple rounds of mapping using the same parameters as stated above, markers with highly distorted segregation or which led to double-crossovers were identified using the function statMark in the ASMap R package and discarded before final map construction. The final genetic linkage map contained 8,869 markers. All markers were analyzed from the initial dataset which had a Hamming distance of 90 with one of the mapped binmap markers were inserted into the map, resulting in a genetic map with 170,719 markers.

Gene annotation and consolidation. We predicted protein-coding structures for F7 and EP1 as consensus models using an approach as previously described. Briefly, consensus gene models are based on transcriptome evidence from F7 and EP1 RNA-seq data generated within this study (see RNA preparation and Expression analysis) and protein homologies of known monocotyledonous protein sequences (maize B73 and PH207, Sorghum bicolor v3.1, Oryza sativa v7.6 and Brachypodium distachyon v3.1) (refs. 59,61). Alignments were found on the genome assembly using GenomeThreader using a minimum alignment coverage of 50% for all sniff-seq sizes 7–10 for protein and 18 for nucleic acid matches. Before mapping, transcript data were assembled using Trinity with default parameters and Bridger with k-mer sizes 25 and 29 bp (refs. 62,63). Subsequently, these sequences were combined by the evidential gene pipeline (http://arthropods.eugenes.org/EvidentialGene) to obtain the final EP1 and F7 transcriptome assemblies. Initial consensus gene models were subsequently consolidated as described in the Supplementary Notes to derive final gene sets for the four flints and two inbred lines.

FISH and karyotyping. Maize chromosomes were prepared from root meristems of 2-day-old seedlings. Roots were cut and treated with 2 mM of 8-hydroxyquinoline solution for 3.5 h. Fixation was performed overnight at room temperature in 3:1 (ethanol:acetic acid) fixative. Slides were prepared according to ref. 64 and in situ hybridization was performed as described in ref. 65. Oligonucleotide probes for karyotyping and identification of knob repeats were chosen from ref. 66. Images were taken using an Olympus BX61 microscope equipped with an ORCA-R2 camera (Hamamatsu). All images were acquired in gray scale and pseudocoloured with Adobe Photoshop CS5 (Adobe Systems). Karyotyping was performed using the ORCA-ER CCD camera (Hamamatsu). All images were acquired in gray scale and subjected to randomization studies and run-of-head statistics, the expected maximal run lengths of identical SNPs between two lines range from 21 to 30 consecutive SNPs. We selected genomic windows of 500 or more SNPs as seeds to identify shared haplotypes of likely common ancestry and used those as seeds for a greedy extend to delineate genomic segments with ≥98% sequence identity between all 15 pairwise line combinations. To deduce higher order haplotypes, we performed the 15 pairwise runs-of-identities to binary SNP patterns and surveyed seeds with ≥40 consecutive identical binary configurations. An iterative greedy algorithm linking the seed with adjacent upstream and downstream runs extended such seeds if the run exhibited a haplotype identical to the seed and less than three.

Whole-genome alignments. Block alignments of high identity were generated for all pairwise combinations of the six maize lines using the MUMMER v.3 suite (refs. 67,68). Initial alignments were computed using numcuv with a minimal cluster size of 250 bp and a seed size of 20 bp. Results were piped through the delta-filter tool selecting the best one-to-one blocks (global option -i) with a minimal size of 500 bp (-s). Despite this filter step, many (41,000–81,000) putatively translocated blocks aligned small regions between different maize genomes on unanchored scaffolds. These blocks frequently overlapped ‘regular’ blocks linking same orthologous chromosomes and showed significantly lower sequence identities in comparison to their respective regular blocks with which they overlapped. Hence, they likely represented paralogous alignments triggered by tandem repeats or the absence of the truly orthologous sequences in one of the lines. To derive the final set of MAbs, we only included candidate interchromosomal translocations with a minimal sequence identity of 99% and a maximum overlap of 100 bp to its adjacent alignments. To gain an overview of the contiguity between the six maize genomes, SABs were connected to MAbs if MAbs were directly adjacent in both genomes with a consistent orientation (Supplementary Fig. 11). All regions identified for each genome were then clustered into genomic regions that aligned (1) to none of the other lines (designated as ‘unaligned’), (2) to all lines of the same group (either flint or dent maize) and to none of the other group (aka ‘group-specific’) or (3) to all five other lines (‘core’), we superimposed SABs and MAbs of one line on its genome coordinates by recording from which group and how many times a single genomic base participated in an alignment. We classified each base and concatenated adjacent bases of the same type (1–3) to derive the unaligned, group-specific and core genomic part. Reversing this approach, identified core regions of EP1 were reprojected onto the pairwise alignments to decode the sequence and coordinates of matching core elements in the other five lines. On the basis of these coordinates, sequences for each core block were extracted and aligned applying the Fast Sequence Aligner with default parameters.

Determination of paired and higher order haplotypes. SNPs were directly determined from multiple sequence alignments of the WGA core blocks (omitting insertions/deletions). Their position in each of the six genomes was derived from the position in the alignment and the known genomic start- and end-positions of the WGA core blocks. Given the observed SNP frequency and using randomization studies and run-of-head statistics, the expected maximal run lengths of identical SNPs between two lines range from 21 to 30 consecutive SNPs. We selected genomic windows of 500 or more SNPs as seeds to identify shared haplotypes of likely common ancestry and used those as seeds for a greedy extend to delineate genomic segments with ≥98% sequence identity between all 15 pairwise line combinations. To deduce higher order haplotypes, we performed the 15 pairwise runs-of-identities to binary SNP patterns and surveyed seeds with ≥40 consecutive identical binary configurations. An iterative greedy algorithm linking the seed with adjacent upstream and downstream runs extended such seeds if the run exhibited a haplotype identical to the seed and less than three.
nonmatching SNPs were observed between the current end of the extension and the candidate run.

RNA preparation. Different tissues of the European maize (Z. mays) inbred lines EP1 and F7 were sampled according to refs. 70, 71 and subsequently subjected to transcriptome sequencing (Supplementary Table 5). Samples 1–3 were collected from seeds imbibed (whole seed, sample 1) or germinated (primary root, sample 2; coleoptile, sample 3) in a 16 h light (28°C) and 8 h dark (21°C) regime of a growth cabinet (Convar CMP6010, http://www.convar.com). Tissue samples 4–24 were taken from plants grown in a climate chamber either in small pots (14 cm top diameter, 8 cm height, 0.251 volume for samples 4–17) or in big pots (28 cm top diameter, 21 cm height, 101 volume for samples 18–24) containing soil substrate type ED 73 (https://www.meyer-shop.com). Growth conditions in the climate chamber were 16 h light (28°C) and 8 h dark (21°C). Two individual plants were collected for each of the 24 samples. The de novo assembled genomes and raw reads were released in collaboration with the Maize Genetics and Genomics Database MaizeGDB18 and NCBI BioProjects PRJNA369023 (F7, DK105 and PE0075) and PRJNA369029 (EP1). Additionally, RNA-seq raw data were also deposited under these NCBI accessions.

Expression analysis and analysis of DEGs. Genomic variants overlapping the flint/dent specific haplotype identified in this study were extracted from the Maize HapMap v3.2.1 panel1 for lines of the 284 inbred maize association panel1. Selected variants were subjected to phylogenetic analysis using FastTree (v.2.1.5 SSE3)39 with default parameters and the resulting phylogenetic tree was visualized using iTOL79 (Supplementary Fig. 18). Two nonoverlapping subtrees were selected such that three of the lines presented in this study (B73, EP1 and F7) are contained, together with other topologically close lines. All lines in the subtree containing B73 were assigned to group one; all those in the subtree containing both EP1 and F7 were assigned to group two.

For the resulting subset of 41 lines (Supplementary Fig. 9) samples from seven different tissues (germinating shoot and root, the tip and base of leaves as well as leaf samples under light and dark conditions and kernels) were downloaded from SRA (PRJNA383416)40. Raw preprocessing was performed as described in the Lexemn Quxent user guide (https://www.lexemn.com/wp-content/uploads/2018/10/015UG10V8201-Quxent-Data-Analysis-Pipeline.pdf). Trimmed reads were mapped to the maize reference sequences B73 (v4), EP1 and F7 using STAR aligner (v.2.5.1a (ref. 80) applying parameters outlierFilterMaxNmatches=10, outlierFilterMismatchNmax=0.04, outlierFilterIntronMotifs=RemoveNoncanonicalUna nnotated To obtain nonunique gene-level counts from the mapping files, HTSeq (v.0.11.2) with the 'nonunique all' method was used41. Normalization of read counts was performed by library sequence depth using the R package DESeq2 (v.1.23.3)42. For differential gene expression analysis with DESeq2, lines classified into either the flint- or dent-like group were treated as biological replicates. In this study, we report only genes for which all three orthologs of RNA-seq read mappings to B73, EP1 and F7 were significant at Benjamini–Hochberg adjusted \( p \leq 0.05 \).

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

Data availability

The de novo assembled genomes and raw reads were released in collaboration with the Maize Genetics and Genomics Database MaizeGDB18 and NCBI BioProjects PRJNA369023 (F7, DK105 and PE0075) and PRJNA369029 (EP1). Additionally, RNA-seq raw data were also deposited under these NCBI accessions.

References

48. Mayer, M. et al. Is there an optimum level of diversity in utilization of genetic resources? Theor. Appl. Genet. 130, 2283–2295 (2017).
49. Avni, R. et al. Wildtype genome architecture and diversity elucidate wheat evolution and domestication. Science 357, 93–97 (2017).
50. Saghai-Maroor, M. A. et al. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl Acad. Sci. USA 81, 8014–8018 (1984).
51. Mascher, M. et al. Anchoring and ordering NGS contig assemblies by population sequencing (P0PSSEQ). Plant J. 76, 718–727 (2013).
52. Taylor, J. & Butler, D. R package ASMap: efficient genetic linkage map construction and diagnosis. J. Stat. Softw. 79, 1–29 (2017).
53. Paterson, A. H. et al. The Sorghum bicolor genome and the diversification of grasses. Nature 457, 551–556 (2009).
54. Wang, W. et al. The Spirodela polyrhiza genome reveals insights into its neotenic reduction fast growth and aquatic lifestyle. Nat. Commun. 5, 3111 (2014).
55. Kawahara, Y. et al. Improvement of the Orzya sativa Nipponbare reference genome using next generation sequence and optical map data. Rice 6, 4 (2013).
56. The International Brachypodium Initiative Genome sequencing and analysis of the model grass Brachypodium distachyon. Nature 463, 763–768 (2010).
57. Gremme, G. et al. Engineering a software tool for gene structure prediction in higher organisms. Inf. Syst. Technol. 47, 965–978 (2005).
58. Chang, Z. et al. Bridger: a new framework for de novo transcriptome assembly using RNA-seq data. Genome Biol. 16, 30 (2015).
59. Grabherr, M. G. et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29, 644–652 (2011).
60. Aliyeva-Schnorr, I., Ma, L. & Houben, A. A fast air-dry dropping chromosome preparation method suitable for fish in plants. J. Vis. Exp. 106, 53470 (2015).
61. Badaeva, E. D. et al. in Fluorescence In Situ Hybridization (FISH): Application Guide (ed. Liehr, T.) 477–494 (Springer, 2017).
62. Zheng, Z. et al. A simple and efficient nondenaturing FISH method for maize chromosome differentiation using single-strand oligonucleotide probes. Genome 60, 657–664 (2017).
63. Spannagl, M. et al. PGSB PlantsDR: updates to the database framework for comparative plant genome research. Nucleic Acids Res. 44, D1141–D1147 (2016).
64. Ellinghat, D., Kurtz, S. & Willhoefl, U. LTRHarvest, an efficient and flexible software for de novo detection of LTR retrotransposons. BMC Bioinformatics 9, 18 (2008).
65. SanMiquel, P. et al. The paleontoge of intergene retrotransposons of maize. Nat. Genet. 20, 43–45 (1998).
66. Benson, G. Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res. 27, 573–580 (1999).
67. Kurtz, S. et al. A new method to compute K-mer frequencies and its application to annotate large repetitive plant genomes. BMC Genomics 9, 517 (2008).
68. Ou, S. & Jiang, N. LTR_retriever: a highly accurate and sensitive program for identification of long terminal repeat retrotransposons. Plant Physiol. 176, 1410–1412 (2018).
69. Xu, Z. & Wang, H. LTR_FINDER: an efficient tool for the prediction of full-length LTR retrotransposons. Nucleic Acids Res. 35, W265–W268 (2007).
70. Ou, S. & Jiang, N. LTR_FINDER_parallel: parallelization of LTR_FINDER enabling rapid identification of long terminal repeat retrotransposons. Mob. DNA 10, 48 (2019).
71. Kurtz, S. et al. Versatile and open software for comparing large genomes. Genome Biol. 5, R12 (2004).
72. Bradley, R. K. et al. Fast statistical alignment. PLoS Comput. Biol. 5, e1000392 (2009).
73. Downs, G. S. et al. A developmental transcriptional network for maize defines coexpression modules. Plant Physiol. 161, 1830–1843 (2013).
74. Sekhon, R. S. et al. Genome-wide atlas of transcription during maize development. Plant J. 66, 553–563 (2011).
75. Hetz, W. et al. Isolation and characterization of rtxs, a maize mutant deficient in the formation of nodal roots. Plant J. 10, 845–857 (1996).
76. Schroeder, A. et al. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol. Biol. 7, 3 (2006).
77. Flint-Garcia, S. A. et al. Maize association population: a high-resolution platform for quantitative trait locus dissection. Plant J. 45, 1054–1065 (2006).
78. Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree 2—an approximately maximum-likelihood trees for large alignments. PLoS Comput. Biol. 9, e1003320 (2013).
79. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–22 (2013).
80. Anders, S., Pyl, P. T. & Huber, W. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166–169 (2015).
81. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550 (2014).

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**Author contributions**

C.-C.S., K.F.X.M., M.O. and G.H. designed the study. K.F.X.M. and G.H. wrote the manuscript. E.B. and C.-C.S. supervised the genomic sequencing. E.B., C.U. and A.N. generated the F2 population and constructed the genetic map. G.H. analyzed genes and whole-genome alignments. H.G. performed the repeat analysis. A.H. and A.R. contributed the FISH analysis. C.M. and F.H. prepared and sequenced the RNA-seq samples. N.K., M.A.S. and I.F. conducted the DEG expression analysis and the phylogenetic analysis. A.S. and M.A.S. provided bioinformatics support. All authors critically read and edited the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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Extended Data Fig. 1 | Assembly sizes and overall repeat content of 8 maize lines. a. Chromosome sizes and N-content (black bars). b. Basic repetitivity in form of 20mer frequencies. Cumulative plot, lower curves represent higher repetitivity e.g. 50% of the PH207 assembly consists of 20mers occurring \( \leq 10 \) times, for the other lines this value is only around 40%. c. Transposon content.
Extended Data Fig. 2 | Genetic versus physical distances for all ten chromosomes of the EP1xPH207 cross. X-axis: physical position on the respective EP1 chromosome in [Mb], Y-axis: genetic position in centiMorgan [cM].
Extended Data Fig. 3 | Group-specific syntelogs for all (4,2)-combinations of the six lines and their syntelog set. We divided the six maize lines of this study into two groups of 4 (gray circles) and 2 (white circles) lines, respectively. For all 15 combinations, the number of group-specific syntelogs for each group was determined under the prior of the identified orthologous gene set. The germplasm-specific separation of flint (EP1, F7, DK105 and PE0075) versus dent (B73 version 4 and PH207) genotypes showed the largest distinction between the two groups.
Extended Data Fig. 4 | Expression levels of orthologs with contiguous and disrupted open reading frame (ORF). Consolidated mappings discovered several thousand genes for which the entire cross-mapping of the top-scoring consolidation model had no contiguous but a disrupted ORF. These genes exhibit highly similar expression levels to their nondisrupted orthologs illustrated here for lines EP1 and F7. a, shows the control correlation and linear regression (red line) of a random sample of F7 and EP1 orthologous pairs for which both genes have a contiguous ORF. To obtain comparable sample sizes and expression ranges, random sampling was restricted to gene pairs with a maximal TPM ≤ 100. b, and c, display expression correlations between gene pairs with exactly one disrupted and one contiguous ORF, while in subfigure d, both orthologous partners contain a disrupted ORF. N: number of pairs, r²: coefficient of determination.
Extended Data Fig. 5 | See next page for caption.
**Extended Data Fig. 5 | Full-length LTR-retrotransposon detection in different maize lines.**

**a**, De novo detection and stringent quality filtering of fl-LTR elements. **b**, Retrieved fl-LTR numbers as proxy for assembly quality. Upper data points give candidate fl-LTRs, lower data points give filtered, high-quality fl-LTR numbers. Five of the six assemblies contain the expected number of fl-LTRs. The by 50% reduced numbers of high-quality elements in PH207 reflect an older not optimized assembly approach. **c**, Insertion age distribution of fl-LTRs. The four NRGene assemblies have very similar age distribution patterns. The B73 assembly has a higher proportion of very young Copia and Gypsy elements, which can probably be attributed to technical assembly differences. In PH207, the young elements are completely missing in the assembly and thus explain the reduced numbers. Due to their (almost) identical long terminal repeats the correct structure of young elements is more difficult to resolve.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Tandem repeat content and composition in the genome assemblies. a, The assemblies of EP1 and F7 captured the highest amounts of tandem repeats and knob sequences. The in spite of high fish intensities much lower contents in the B73 and PH207 assemblies reflect the assembly difficulties of these highly repetitive genome regions. b, Relationship between the six lines derived from knob location similarities. The input matrix for clustering contained three relative intensities of fish knob locations per chromosome. The knob patterns of the two Dents B73 and PH207 are very similar, as well as the patterns of DK105 and PE0075. c, Multiple sequence alignment of selected knob monomers from EP1 together with known knob monomers. The knob sequences in the EP1 assembly consist of 180 and 202 bp monomers with a surplus of the 180 bp monomer by a factor of 6.8. Both monomers are highly similar to previously reported knob monomers from maize with the following Genbank IDs marked as 1, 2, 3 in the figure, 1: AF030934.1, 2: M325211 and M325251, 3: DQ352544.1_a and DQ352544.1_b. Consensus sequences of the monomers where used to identify all major and minor knob locations in the assemblies. d, Gene expression (maximal expression of 7 different conditions per Gene, log10) in relation to the nearest upstream knob signature (left) and tandem repeat (right). Both axes are logarithmic.
Extended Data Fig. 7 | Syntenic knob signatures. x-axis: number of knob units per 2 Mb windows, y axis: chromosome length in Mb. a, Minor knob locations in the assemblies (y max=100). The majority of the minor knob locations are syntenic between the 8 lines, especially for chromosomes 4, 5, and 6, and display no clear distinction between flint and dent. This is in contrast to the knob intensities and extent detected by FISH hybridizations (Fig. 3a). We hypothesize that these minor locations act as ‘anchor points’ for knob movement or expansion, which seem to occur within very short evolutionary time-spans since the separation of different breeding lines. Larger knob stretches could impact the expression of surrounding genes and influence local recombination rates. b, Major knob locations in the assemblies (Y max=4000).
Extended Data Fig. 8 | Densities of the core, group-specific and unaligned regions for the six maize lines studies. Intensities are normalized within one track, dark red/blue values representing a higher fraction of aligned regions per 5 Mb bins. Outer chromosome ideograms show the largest of all six lines, dark gray ellipses indicate the approximate centromere position derived from the repeat analysis. Group of six circular tracks show from outer to inner the core, germplasm-specific and unaligned densities. Each group consists of six tracks, displaying from outer to inner densities for B73, PH207, EP1, F7, DK105 and PE0075.
Extended Data Fig. 9 | Genomic sizes in [Mb] of SNP runs-of-identity (RoI) for all 15 pairwise comparisons of the six maize lines. Comparisons within one germplasm (flint: EP1, F7, DK105 and PE0075; dent: B73 version 4 and PH207) share larger genomic fractions than inter-germplasm RoI totals. Y-axis: total sum of RoIs in [Mb], X-axis: line pairs, gray circles indicate maize line used in the respective comparison.
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| Illumina HiSeq 2500 and 4000 produced genomic and RNAseq paired-end reads, respectively; |

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Data has been released as NCBI bioproject PRJNA360923 and PRJNA360920. Genome sequences and gene models (GFFs) are also available at maizeGDB (https://www.maizegdb.org)
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