The mosaic oat genome gives insights into a uniquely healthy cereal crop

Cultivated oat (Avena sativa L.) is an allohexaploid (AACCDD, 2n = 6x = 42) thought to have been domesticated more than 3,000 years ago while growing as a weed in wheat, emmer and barley fields in Anatolia1,2. Oat has a low carbon footprint, substantial health benefits and the potential to replace animal-based food products. However, the lack of a fully annotated reference genome has hampered efforts to deconvolute its complex evolutionary history and functional gene dynamics. Here we present a high-quality reference genome of A. sativa and close relatives of its diploid (Avena longiglumis, AA, 2n = 14) and tetraploid (Avena insularis, CCDD, 2n = 4x = 28) progenitors. We reveal the mosaic structure of the oat genome, trace large-scale genomic reorganizations in the polyploidization history of oat and illustrate a breeding barrier associated with the genome architecture of oat. We showcase detailed analyses of gene families implicated in human health and nutrition, which adds to the evidence supporting oat safety in gluten-free diets, and we perform mapping-by-sequencing of an agronomic trait related to water-use efficiency. This resource for the Avena genus will help to leverage knowledge from other cereal genomes, improve understanding of basic oat biology and accelerate genomics-assisted breeding and reanalysis of quantitative trait studies.

Genome assembly and composition

We produced a chromosome-scale reference sequence of oat cv. ‘Sang’ comprising 21 pseudochromosomes (Fig. 1, Extended Data Fig. 1a and Supplementary Table 1), with a BUSCO (v5.1.2; ref. 6) score of 98.7% (Extended Data Fig. 2a), following the short-read strategy used for wheat7, barley8 and rye9. Inspection of Hi-C contact matrices (Supplementary Fig. 1) and the consensus genetic map 10 (Supplementary Fig. 2a) and their comparison with the independent assembly

Oat is a member of Poaceae, an economically important grass family that includes wheat, rice, barley, common millet, maize, sorghum and sugarcane. Avena species exist in nature as diploids, tetraploids and hexaploids and exhibit the greatest genetic diversity around the Mediterranean, Middle East, Canary Islands and Himalayas. Currently, oat is a global crop with production ranking seventh among cereals (http://www.fao.org/faostat/en/, accessed May 2021). Compared with that of other cereals, oat cultivation requires fewer treatments with insecticides, fungicides or fertilizers. Whole-grain oats are a healthy source of antioxidants, polyunsaturated fatty acids, proteins and dietary fibre such as β-glucan, which is important in post-meal glycaemic responses and for preventing cardiovascular disease3–5. Cereals such as wheat, barley and rye store high amounts of gluten proteins in their grain; by contrast, oat and rice store globular proteins in their grain.

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markers (d) distribution of high-confidence genes (repeat retrotransposons) on chromosomes (dark red). Figure generated with Circa (http://omgenomics.com/circa).

The overall structure of the oat genome is similar to that of Triticeae genomes, although frequent genomic rearrangements in oat have resulted in a mosaic-like genome architecture. In many oat chromosomes, gene and recombination density is not a monotonic function of distance from the centromere (Extended Data Fig. 3), as is mostly observed in the Triticeae 25. Examination of whole-genome alignments, subgenome-specific k-mers and orthologous and homoeologous genes clustering as syntenic blocks in genomic neighbourhoods in four Avena species (Extended Data Figs. 1d and 4) revealed numerous large-scale genomic rearrangements affecting the order of these blocks within and between subgenomes (Fig. 2a). We detected seven large-scale genomic rearrangements in A. sativa and traced them back to eight translocation events between the A, C and D subgenomes (Fig. 2b, c, Extended Data Figs. 4a and 5a, Supplementary Fig. 10 and Supplementary Table 8), spanning 4.3% of the genome and approximately 7.9% of the high-confidence genes. Two of the translocation events were specific to A. sativa. Unlike those in wheat, the oat subgenomes exhibit unbalanced gene counts; specifically, the C subgenome appears to have 12 fewer genes than the A or D subgenome (Extended Data Fig. 2d and Supplementary Table 9). Analysis of orthologous gene groups (Supplementary Table 10 and Supplementary Figs. 11 and 12) showed that unbalanced gene families were associated with significant spatial clustering (Supplementary Fig. 13) in genomic rearrangements. Ancestral state reconstruction of the oat chromosomes revealed a loss of at least 226 Mb of gene-rich regions from the C subgenome to the A and D subgenomes (Supplementary Table 9). This implies that the translocations fully account for the lower gene count in the C subgenome and not gene loss or subfractionation after formation of the hexaploid.

Previous molecular marker studies using oat mapping and breeding populations have provided independent evidence for frequent translocations among oat subgenomes 26-28. Using the oat genome to reanalyse the data (Extended Data Fig. 6), we observed inter-chromosomal pseudo-linkage in a population that segregates for the IC translocation on 1A. Such pseudo-linkage has been implicated in the propensity for cold hardiness to remain associated with non-carriers of this translocation 29. An accompanying study 30 details similar associated opportunities and barriers in genomic breeding strategies. The mosaic nature of the oat genome may be associated with the apparent lack of an orthologue of TaZIP4-B2 (located within the Phl locus), which in bread wheat stabilizes the genome structure during meiosis and suppresses crossovers between homoeologues 31-34 (Extended Data Fig. 5b and Supplementary Figs. 14 and 15). In contrast to wheat, interploidy crosses and alien introgressions have been extremely challenging in Avena 35, suggesting that incompatible genome architecture is an additional barrier preventing genetic gains in oat.

**Mosaic chromosome architecture of oat**

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Oat subgenome expression is balanced

After polyploidization, sub- and neofunctionalization and gene loss modify the gene content in the new species27,28. Systematic differences in subgenome/homoeologue gene expression (homoeologue expression bias) may also be prevalent. In fact, quantitative variation for many agronomic traits may reflect genetic interactions between homoeologues such as functional redundancy (buffering) or dominant phenotypes attributed to one homoeologue46. To investigate homoeologue expression bias in hexaploid oat, we defined 7,726 homoeologous gene triads with a 1:1:1 correspondence across the three oat subgenomes (Supplementary Table 11), referred to as ancestral triads. Average expression values across transcriptome samples from six tissues showed that C-subgenome genes were slightly less expressed (32.32%) than those in the D (33.53%) and A (33.76%) subgenomes (Kruskal–Wallis, *P = 0.054). We considered six homoeologue expression categories30 and found that most ancestral triads (84.1%) showed balanced expression, 3.4% showed single-homoeologue suppression and 12.6% showed single-homoeologue suppression. The relative contributions of the different categories (Extended Data Fig. 7a) indicated no major overall bias for one of the subgenomes (Fig. 2d).

A co-expression network approach revealed that genes from the C subgenome were found in divergent expression modules more frequently than their A- and D-subgenome homoeologues (χ² test, *P = 2.085 × 10⁻⁶; Extended Data Fig. 7b and Supplementary Table 12).

In another 1,508 triad gene clusters containing at least one member positioned in a translocated region (relocated triads; Supplementary Table 13), the overall expression patterns were similar to those of the ancestral triads (Extended Data Fig. 7c). The C-suppressed category was slightly larger (5.1%) in the ancestral triads compared with the A-suppressed (3.5%) or D-suppressed (4.1%) triads, but the subgenome suppression patterns were reversed (4.5% A, 4.2% C and 5.2% D) in the relocated triads (χ² test, *P = 0.019; Extended Data Fig. 7c). Our results indicate that translocations and rearrangements in the oat genome may affect global and homoeologous gene expression patterns. Understanding how homoeologues interact
Mixed-linkage β-glucans are soluble fibres present at high levels in oat endosperm cell walls (3.8–6.1 g per 100 g dry weight) that reduce blood cholesterol and post-meal glycaemic responses. The cellulose synthase-like gene \(CslF6\) is central for β-glucan biosynthesis in cereals. We catalogued the cellulose synthase (\(CesA\)) and callose synthase (\(GT48\)) families of glycosyltransferases to identify the genetic foundation underlying oat β-glucan biosynthesis. The hexaploid oat genome encodes 134 members of the cellulose synthase gene superfamily (Fig. 3a), representing the cellulose synthase (\(CesA\)) subfamily (Supplementary Fig. 16) and ATIs. Soluble fibre-related gene families

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seven cellulose synthase-like subfamilies, including CslA, CslC, CslD, CslE, CslF, CslH and CslJ. The G748 family comprised 28 members (Supplementary Fig. 17). Genes within the CesA and CslF subfamilies were most highly expressed over multiple stages of seed development (Fig. 3a and Supplementary Fig. 18). Investigation of differentially expressed genes between stages indicated specific roles for particular subfamilies such as CslE and CslF (including the C-subgenome copy of CslF6), which were upregulated in late stages of seed development (Supplementary Fig. 19), as shown in barley34. Compared with other grasses, the oat cellulose synthase superfamily showed no significant expansions apart from duplication events in the CesA, CslC, CslE and CslF subfamilies (Supplementary Fig. 20 and Extended Data Fig. 8). These findings suggest that the high content and quality of β-glucan in oat are not driven by major differences in the copy number of cellulose synthase superfamily genes relative to other grasses but rather by allelic variation and transcription factors, as previously reported34.

### Oat storage proteins and human health

Oat globulins constitute 75–80% of grain protein content, with prolamin (avenins) accounting for approximately 10–15%. Prolamin
superfamily members trigger coeliac disease, food allergies and baker’s asthma. We identified genes encoding 25 avenins, 6 high-molecular-weight glutenins (HMW-GS) and 61 genes representing α-amylase/trypsin inhibitors (ATIs) and other prolamin superfamily members related to protein accumulation and immunogenicity (Supplementary Table 14). Hexaploid oat has avenin loci on chromosomes 1D, 3D and 7A; seed storage globulin loci (135 genes) on chromosomes 1A, 1D, 3D, 7A, 4A and 4D; and no storage protein loci mapping to the C subgenome (Fig. 3b and Supplementary Table 14).

Unlike that of wheat, the oat genome harboured no α- or ω-gliadin genes, and the identified avenins co-clustered with γ-gliadins, low-molecular-weight glutenins and B-hordeins (Fig. 3b). We detected four complete, highly conserved oat HMW-GS gene models as two distinct loci on 1A and one locus pair on 1D, with no HMW-GS genes mapping to IC. We identified a prolamin type, the 19-kDa gliadin-like proteins, with an unknown function that is distinct from the avenins yet shares sequence similarity with HMW-GS and 19-kDa glioblins (Fig. 3b). The predicted oat HMW-GS and avenins were highly conserved in their Pfam domains (Fig. 3b) and cysteine peptides (Extended Data Fig. 9). Glutamine- and proline-rich repetitive peptides were fewer in these oat proteins, making them shorter than those in wheat or barley (Extended Data Fig. 9).

We detected transcripts for most of the avenin genes, which showed gene expression patterns that aligned with their wheat orthologues, with increased transcript levels from the middle phase of seed development (Fig. 3c), and protein levels by using liquid chromatography with tandem mass spectrometry (Fig. 3b). We identified inactive genes and pseudogenes among avenin-encoding genes (Fig. 3b, c and Supplementary Table 14) in a similar proportion as in wheat γ-gliadins. This indicates a lower level of gene expansion and pseudogenization compared with the highly immunogenic wheat α-gliadin genes. Moreover, the expression of 11S globulin genes initiated early in seed development and was higher than that of the avenin genes (Fig. 3c). Discovery proteomics detected thirty-six distinct 11S globulins, five globulin-1 proteins and two 7S globulins, with an average of 83% protein sequence coverage at a 1% false discovery rate.

The oat avenins and globulins showed opposite trends compared with their wheat orthologues in gene copy number, protein length and enrichment in glutamine and asparagine residues that serve as a nitrogen storage sink (Extended Data Fig. 10a). Together with pronounced differences in transcription factor-binding sites specific to the nitrate response (Extended Data Fig. 10b and Supplementary Table 15), this may contribute to the primary role of oat globulins in nitrogen storage. These results confirm that the genomic organization, sequence characteristics and expression patterns of oat storage proteins share more similarities with rice and dicotyledonous plants than with wheat and other gluten-rich cereals.

We mapped previously reported coeliac disease-associated T cell epitopes to the predicted oat avenin proteins and compared them with the T cell epitope patterns of wheat and barley prolamins. The results showed that only a subset of encoded avenin proteins contain coeliac disease-associated immune-reactive regions compared with the high prevalence found in wheat or barley (Fig. 3b). Taken together, the low copy number of genes encoding coeliac disease epitopes, low frequency of detected T cell epitopes in the protein sequence, low occurrence of other highly immunogenic proteins, proportion of avenins within total oat protein and relative immunogenicity of avenin epitopes all support the inclusion of oats in gluten-free diets.

Single-gene mapping of a wax mutant
To demonstrate how an annotated reference genome enables greater use of resources such as TILLING populations, we mapped the causal mutation in the epicuticular wax mutant glossy J (Fig. 4a, b). Epicuticular waxes have a role in biotic and abiotic stress resistance and provide an important target for oat breeding. We identified homozygous polymorphisms unique to the mutant, which mapped to chromosome IC (Fig. 4c and Supplementary Fig. 21), and identified a single gene annotated as an α/β-hydrolase (AVESA.00010b.r2.UnG1403470) as a likely candidate that is orthologous to barley Cer-q (HORVU.MORE Xr3.2HG0097460) (Supplementary Fig. 22). An independent mutant line (glossy-2) exhibited the same glossy phenotype (Fig. 4d, Supplementary Fig. 23 and Supplementary Table 16). Barley Cer-q mutants are deficient in the same β-diketone (hentriacontane-14,16-dione) and wax tubules that are absent in the glossy mutants (Fig. 4e–g and Supplementary Figs. 24–26). The scaffold containing the candidate gene was localized to the region of chromosome IC (Fig. 4c and Supplementary Table 17). The presumed glossy J mutation introduced a P243S substitution in the encoded protein adjacent to a deleterious F219L substitution known to inactive barley CER-Q (4d) and Supplementary Fig. 27). We identified gene clusters on oat chromosomes IC, 2C and 3A and in wild Avena species (Supplementary Figs. 22 and 28–33) that are homologous to the barley Cer-cqu cluster. We also noted genes encoding proteins with similarity to Arabidopsis wax ester synthase/diacylglycerol acyltransferase 1 (WSD1), a Myb-domain transcription factor and a short-chain dehydrogenase/reductase (SDR) protein near the Cer-cqu homologues in the Avena genomes. All genes from the IC cluster except SDR were expressed at levels 3–6 times higher than those of the 3A cluster, with very low expression from 2C cluster genes and with no differential expression between the glossy and glaucous glume tissue (two-sided Wald test, null hypothesis logarithmic fold change = 0, adjusted P < 0.01; Fig. 4h and Supplementary Fig. 34). Together, these results suggest that AVESA.00010b.r2.UnG1403470 is the oat Cer-q gene. The reference genome thus facilitated a major advance in understanding β-diketone biosynthesis in oat and can help breeders manipulate tissue-specific epicuticular wax composition in future oat cultivars adapted for hotter climates.

Discussion
In summary, this fully annotated hexaploid oat reference genome lays the foundation for advances in oat breeding and basic oat biology and for the ongoing pan-genome project. With the chromosome rearrangements in a typical spring oat cultivar now delineated, breeders and researchers will have access to a resource equal in calibre to Triticeae genomes, which may help them to overcome the breeding barriers and segregation anomalies described in numerous mapping studies. Using the reference genome to map genes associated with agronomic and human nutrition-related traits is a viable approach for precisely adapting oat varieties. Known quantitative trait loci can be anchored to the Sang reference, and the transcriptome atlas co-expression networks can be leveraged to identify candidate genes in the vicinity of specific quantitative trait loci. Modern breeding strategies such as genome editing and gene pyramiding can now more easily be applied in oat to develop varieties that meet the increasing global demand for oat-derived products. Our proteogenomic investigation of oat storage proteins confirms qualitative and quantitative differences in the expression of proteins compared with the more abundant and immunogenic sequences in wheat, barley and rye, which supports the safety of oats in gluten-free diets. The detailed genome annotation and case studies presented here provide examples of the myriad possibilities for the discovery and exploitation of functional genetic mechanisms in oat.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-022-04732-y.
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Article

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

Data availability
The raw sequence data used for de novo whole-genome assembly are available from the European Nucleotide Archive (ENA) under accession number PRJEB44810 (A. sativa cv. Sang) and from the Sequence Read Archive under accession numbers PRJNA27490 (A. insularis BYU209) and PRJNA726919 (A. longiglumis CNS3183). Chromosome conformation capture (Hi-C) sequencing data are available from ENA under accession numbers PRJEB43668 (A. sativa cv. Sang), PRJEB43670 (A. insularis BYU209) and PRJEB43669 (A. longiglumis CNS3183). Chromosome-scale sequence assemblies (pseudomolecules) are available from ENA under accession numbers PRJEB44810 (A. sativa cv. Sang), PRJEB45088 (A. insularis BYU209) and PRJEB45087 (A. longiglumis CNS3183). The raw RNA-seq and genome-sequencing data generated in this study are available under ENA accession number PRJEB46365. Pseudomolecules, annotation data and analysis results are available at the Plant Genomics and Phenomics Research Data Repository at https://doi.org/10.5447/ipk/2022/2. The DOI was registered using eIDAl (https://edal.ipk-gatersleben.de/). Pseudomolecules, annotation data and associated analyses for A. sativa cv. Sang, A. longiglumis and A. insularis are also available from GrainGenes: Sang genome browser, https://wheat.pw.usda.gov/jb/?data=ggds/oat-sang; Sang data download, https://wheat.pw.usda.gov/GG3/content/avena-sang-download; A. longiglumis genome browser, https://wheat.pw.usda.gov/jb/?data=ggds/oat-longiglumis; A. longiglumis data download, https://wheat.pw.usda.gov/GG3/content/avena-longiglumis-download; A. insularis genome browser, https://wheat.pw.usda.gov/jb/?data=ggds/oat-insularis; A. insularis data download, https://wheat.pw.usda.gov/GG3/content/avena-insularis-download. The mass spectrometry proteomics data and ProteinPilot search result files have been deposited to MassIVE (https://massive.ucsd.edu) under accession number MSV000088727. The publicly available OT3098 oat genome data were generated by PepsiCo and Corteva Agriscience. This dataset (annotation version 2) has been obtained and is available from GrainGenes at https://wheat.pw.usda.gov/GG3/content/pepsi-oatct0308-hexaploid-oat-version-2-genome-assembler-release-collaboration-grainenes. Databases used in this study included TRAP release 19, Uniref download 2019-09-03, Pfam download 2019-09-03, Swiss-Prot, TAIR, TrEMBL, Reda_9.9_Poaceae section of the PGSB transposon library, Immune Epitope Database and Analysis Resource (https://www.iedb.org), PLACE and PlantCare promoter motif databases and pfam2GO.

Code availability
All relevant code developed in this study was deposited in the GitHub node at https://github.com/PGSB-HMGU/oatkmers.

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Author contributions
O.O. conceived the Sang genome study and secured funding. N. Sirijovski and M.S. conceived and coordinated the study. N. Sirijovski performed molecular biology and coordinated sequencing and assembly for Sang. N.A.T., W.A.B., E.N.J., P.J.M. and Y.-B.F. contributed A. insularis and A. longiglumis assemblies. A.H. and N. Stein prepared Hi-C libraries and performed sequencing. M.M. performed pseudomolecule assemblies. M.M. and H.G. determined chromosome nomenclature. N. Sirijovski prepared RNA for atlas samples presented in this work. A.C. performed sequence data processing. RNA-seq, hexaploid markers and pedigree background. A.I.W. performed Iso-seq data and was principal investigator of the OT3098 genome project. M.S. and K.F.X.M. supervised annotation, with assistance from N.K., N.T.R. and T.L. A.C. performed storage protein analysis; J.A.T.-D. performed storage protein data interpretation. E.N.J. contributed cytogenetic data. N.K., N.T.R. and T.L. performed confidence classification. TE and repeat analyses were performed by H.G. Ontology annotations were provided by D.L., N.V.G. and R.R. J.B. was responsible for data management and submission, with assistance from T.L. N. Sirijovski performed experiments with mutants with support from M.H. and P.S. for profiling of wax metabolites. N.T.R. performed variant calling and single-genome mapping. N.K. conducted expression analysis with assistance from J.B. N.A.T. and W.A.B. performed molecular marker and recombination landscape analysis. E.N.J. contributed cytogenetic data. N.K., N.T.R. and A.J. performed gene family analysis and manual annotations. G.H. and H.G. analysed genome structure, organization and translocations. A.I.J. and M.L.C. supervised proteomics. U.B. performed proteomic data acquisition; A.J.I. and J.A.T.-D. performed storage protein data interpretation. M.S. and N. Sirijovski drafted the manuscript with input from N.K., N.T.R., J.B., M.M., A.J., U.B., J.A.T.-D., M.L.C., H.G., and N.A.T. All co-authors contributed to and edited the final version.

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Competing interests
N. Sirijovski was an employee of CropTailor AB, a commercial enterprise, and became employed by Oatly AB during manuscript revision. O.O. is an employee of CropTailor AB. A.C. is an employee at Lantmännen. A.J.W. is an employee of PepsiCo, Inc. These authors are not expected to benefit financially from publication of these results. All other authors declare no competing interests.

Additional information
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Extended Data Fig. 1 | Assembly of three Avena species and chromosome organisation of hexaploid oat. a, Summary of assembly statistics of the hexaploid A. sativa cv. Sang, diploid A. longiglumis CN58138 and tetraploid A. insularis BYU209 genomes. b, Gene-based collinearity of oat (A. sativa) to barley (H. vulgare). Each data point is an aligned gene. Genes in core regions are shown in red. The phylogenetically informed nomenclature is used on the bottom x-axis. The top axis shows the chromosome names according to Bekele et al. (2018)10, (Mrg groups) and according to Sanz et al. (2010)13, respectively. Centromere positions in oat and barley are indicated by red triangles (x axis) and red diamonds (y axis). c, Subgenome composition of hexaploid oat cv. Sang. Subgenome-specific features related to independent transposon histories in the diploid ancestors. The upper part shows the x-fold increase compared to the lowest of the three values across subgenomes A–C. The lower part summarises differences in genome size and gene number between the extant hexaploid and the ancestral state predating the seven translocations between the tetraploid and hexaploid subgenomes. d, Tandem repeat families in hexaploid oat cv. Sang specific to either the C or the A/D diploid ancestor or enriched in the extant A subgenome (from top to bottom).
Extended Data Fig. 2 | Validation and statistics of *A. sativa* cv. *Sang* assembly and gene annotation. **a**, BUSCO (lineage *poales_odb10*, created 2020-08-05) scores of the genome assemblies of *A. sativa* cv. *Sang*, *A. insularis* and *A. longiglumis* as well as the diploid progenitors *A. eriantha* and *A. atlantica* as a comparison. **b**, Colinearity plot of the pseudomolecules of *A. sativa* cv. *Sang* (short read assembly, y-axis) and OT3098 (long read assembly, x-axis). **c**, BUSCO (lineage *poales_odb10*, created 2020-08-05) scores for the oat reference gene annotation v1.1 in comparison to the results of recent gene predictions for maize, rice and bread wheat. **d**, Top: overview of the number of predicted genes (HC - high confidence; LC - low confidence) for the three oat subgenomes and unplaced/unknown scaffolds. Bottom: total number of basepairs assembled for the respective three subgenomes and the number of basepairs in unplaced scaffolds.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Chromosome architecture of hexaploid oat.
Distribution of main features along the 21 Sang chromosomes. Track 1 displays syntenic blocks based on the chromosome pattern of gene clusters with exactly three members. Track 2 denotes the sub genome assignment. The background of the bottom part consists of a stacked bar chart for the major genome components. The lines in the front show smoothed chromosomal distributions for mean 20-mer frequency, average recombination rate (cM/Mb) and gene density (#/Mb), each scaled min to max per chromosome. The population-level analysis of crossover frequency is explored in (N.T., W.B. et al. 21). Therein we focus on average recombination rates that may result from global chromosome restructuring within Avena. Of particular interest are the long stretches of increased recombination on the long arms of chromosomes 4A and 4D. These regions coincide with stretches of high gene density and reduced transposon frequency. Other regions containing multiple ancestral telomeric regions showed corresponding multiple peaks of gene density and recombination. Also of interest is a region of suppressed recombination on chromosome 7D which is speculated to result from intraspecific rearrangements on this chromosome that impede meiotic pairing and crossovers.
Extended Data Fig. 4 | Mosaic composition of hexaploid oat chromosomes.

a. Subgenome-specific kmers and syntenic blocks in *A. sativa*. Probabilities of A- (blue), C- (yellow) and D- (green) subgenome classification by subgenomic kmers are shown. Top row in each subplot displays identified synteny shown in alternative colours to emphasise block borders. From top to bottom, chromosomes 1 to 7 for subgenomes A (left column), C (mid column) and D (right column), Y-axis: kmer-probability, x-axis: chromosomal position in Mb.

b. Ancestral subgenome origin. In the extant Sang genome seven peripheral regions ranging from 40 Mb to 106 Mb differ in their subgenome signature from their current location. Five of these regions are of C genome origin: chr1A (106 Mb), chr2D (40 Mb), chr3D (79 Mb), chr4D (46 Mb), chr5D (62 Mb). Two regions on the extant C subgenome are of D genome origin: chr1C (40 Mb) and chr4C (67 Mb). All in all, the transfers between subgenomes add up to 441 Mb, with a net loss in the C subgenome of 226 Mb. The upper tracks display homeoeologous syntenic blocks within the Sang genome based on the chromosome pattern of gene clusters with exactly three members. The pattern A1-A1-D1 corresponds for example to a cluster with two members from chr1A and one member from chr1D. The core denotes all seven symmetric cluster patterns from A1-D1-C1 up to A7_D7_C7 with one member on each homoeolog chromosome (equivalent to the red dots in Extended Data Fig. 1b).
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Genome rearrangements in oat. a, Translocation history in A. insularis and A. sativa cv. Sang. Top panel displays likely ancestral syntenic blocks for Avena chromosomes 1, 2, 3 and 6, for chromosomes 4, 5 and 7 we only provide ancestral states in the A-, D- and C-lineages separately. From top to bottom, subsequent panels show chromosomes and translocation operations for the initial tetraploid (directly after the hybridization of the diploid ancestors), the ancestral tetraploid after translocations, translocation events occurring after the split of A. insularis and oat, and the extant states. Genomic positions for each inter-subgenomic translocation in the extant tetra- and hexaploid are provided in Supplementary Table 8. A-, C- and D-subgenomic blocks are coloured in blue, yellow and green, respectively. Blocks contributing to translocations are accented in the mid panels by darker colours. Circled numbers represent translocation events: (1) represents two non-reciprocal translocations between the ancestral 1C and 3D chromosomes, (2) to (5) are additional translocations shared by A. sativa and A. insularis, while (6) and (7) are translocation differentiating extant oat and A. insularis. Asterisks refer to the ancestral state of chromosomes. b, Schematic representation of syntenic genomic regions between bread wheat (chromosome 5B harbouring TaZIP4-B2) and oat (chromosomes 5A, 5C, 5D). At the syntenic position, a TaZIP4-B2 ortholog is absent in the oat genome.
Extended Data Fig. 6 | An example of a hidden breeding barrier in *Avena sativa* revealed by recombination matrices in two oat populations. Average pairwise recombination frequencies (r) among chromosomes 1A, 1C and 1D were computed in 16 Mbp windows at 1 Mbp increments for two RIL populations and are visualised as blended colours of yellow (r = 0) to cyan (r = 0.25) to burgundy (r = 0.5). Blocks of yellow along the diagonal dashed lines indicate recombination suppression within a chromosome. Blocks of yellow off the diagonal represent pseudo-linkage between chromosomes. 

**a**, recombination in the population ‘Goslin’ x ‘HiFi’, where Goslin carries a 1C-A translocation (like Sang) and HiFi is a non-carrier. The result is that a large proportion of 1C shows pseudo linkage to the part of 1A where the translocation is present (large yellow rectangle in the intersection of 1A and 1C). 

**b**, both parents of the population (‘TX07CS-1948’ x ‘Hidalgo’) carry the same translocation as Sang thus no pseudo linkage is observed. The 1C-1A translocation is associated with adaptation-related traits, with pseudo linkage limiting the possibility of recombining different traits affected by genes in the translocated region in crosses between translocated and non-translocated germplasm.
Extended Data Fig. 7 | Gene expression analysis in the hexaploid oat genome.

**a**, Box plots representation of the relative contribution of each subgenome based on triad assignment to the seven categories defined, balanced n = 6,308, A-suppressed n = 263, C-suppressed n = 378, D-suppressed n = 303, A-dominant n = 83, C-dominant n = 98, D-dominant n = 74, n = number of triads. Values from the first to third quartiles are shown within the boxplots (inter-quartile range) with the median represented by the middle line. The upper and lower whiskers extend from the edge to the largest and smallest value of the edge but no further than 1.5 × the inter-quartile range, the data beyond the end of the whiskers are outliers plotted individually.

**b**, Heatmap representation of WGCNA modules showing the relation between the expected representation of each subgenome in the module based on the overall number of genes per subgenome and the observed one. > 1: higher than expected, < 1 lower than expected, 1 = as expected.

**c**, Gene expression patterns in ancestral triads versus triads with genes positioned in translocated regions across all samples (upper two panels) and in seed tissue (lower two panels).
Extended Data Fig. 8 | Phylogeny of the CesA-subfamily of cellulose synthase genes and the Cellulose synthase-like subfamilies. CesA, C, D, E, F, H, and J in 11 different species including oat. Tree branches for different subfamilies are shown in different colours. Branch thickness corresponds to bootstrap values and increases with higher bootstrap.
Extended Data Fig. 9 | Comparative analysis of coeliac disease and allergy related epitopes in oat, wheat and barley. a, Protein sequence alignment of avenins with γ-gliadins, B-hordeins, and LMW glutenins shows the conserved position of cysteine residues (black bars) and differences in the T cell epitope prevalence and variability. Blue bars represent T cell epitopes characteristic at γ-gliadins, green bars indicate epitopes characteristic at LMW glutenins, pink bars show positions of avenin-specific epitopes. Colour code on the left side indicates the species: oat (highlighted in pale yellow); wheat (highlighted in pale blue); and barley (highlighted in jagged ice). b, Sequence alignment of HMW glutenins show the conserved position of cysteine residues in the N- and C-terminal regions in oat and wheat HMW glutenins and barley D-hordein and shows the complete absence of coeliac disease (highlighted in red) and wheat allergy-related epitopes (shown as blue bars) in the sequences.
Extended Data Fig. 10 | Comparative analysis of nitrogen storing capacity and transcription factor binding site motifs. 

a, Size distribution of prolamin and globulin protein sequences in amino acid residues (aa) identified in the genomes of hexaploid oat cultivar Sang, the IWGSC v1 wheat reference genome, *Oryza sativa* Japonica IRGSP-1.0 rice genome and soybean reference genome, *Glycine max* v2.1 in relation to their nitrogen storing capacity, measured as a sum of asparagine and glutamine content in their sequence.

b, Comparison of nitrogen-responsive transcription factor binding site patterns in oat, wheat and rice prolams and oat, wheat, rice and soybean globulins. Consensus motifs identified in the 0 to −500 region and the related transcription factor families are labelled in each column; total numbers of detected motifs normalised by the number of gene models are indicated in each row.
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Software and code

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Data collection

Custom code developed in this study can be found in the GitHub repository at https://github.com/PGSB-HMGMU/oatkmers. Code developed in other studies (but used here) can be found at https://github.com/PGSB-HMGMU/plant.annot. Software for data analysis included HiSeq Control Software HD 3.4.0.38/RTA 2.7.7, NovaSeq Control Software 1.7.0/RTA v3.4.4, and bcftools v2.20.0.422.

Data analysis

A myriad software was used in this study, all of which have been listed and cited. These include DeNovoMAGIC v3.0, TRITEX pipeline, EMBASSY restrict, Minimap2, SAMAtools, Novosort (http://www.novocraft.com/products/novosort/), BEDTools, FASTQC v0.11.8, Canu v1.9, Arrow from the Genome Consensus package in the Pacific BioSciences SMRT portal v5.1.0, PIPO v0.22, BBDuk (https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/), cactus pipeline v1.0, tallymer subtools from the genome tools package v1.6.1, GenomeThresher, v1.7.1, HISAT2 v2.1.0, Stringtie v1.2.3, Transdecoder v3.0.0, BLAST+, gffread v0.11.6, Biopython, Diamond v0.9.25 and v0.9.29.130, STAR, v2.7.6a, Trinity v2.8.5, PASA Pipeline v2.3.3 and v2.4.1, GMAP v2017.11.15 and v2020.06.01, TransDecoder v5.6.0, HMMER v3.1.1 and v3.2.1, Augustus v3.3.1 and v3.3.3, Evidence Modeler v7.35.0, BUSCO v3.0.2, iliosipsa_odb10 created on 2017-12-01 and embryophyta_odb9 created on 2017-02-13 (protein mode), BUSCO v4.0.4, iliosipsa_odb10 created on 2019-11-20 (protein mode), AhR v3.3.3, Tandem Repeats Finder v4.07b, vmatch dbcluster, R, haplotag, FSL as implemented in TASSEL v5.0, KMC tools v3, Louvain algorithm (https://github.com/taynaud/python-louvain), Orthofinder v2.4, Scipyr v1.6.1, networkx v2.5, InterProScan5, CLC Genomics Workbench v2.1, ggpubr, FileMaker Pro Advanced v17, MEME suit, Morpheus R package, ProteinPilot v5.0.3 software (SCIEX), MiScan of the jow utility library (https://github.com/funghalban/jowl), MUSCLE v3.8.155, fasttree v2.1.10 and v2.1.11, iTol v6.3, Trimomatic, fastp v0.20.0, Salmon v1.1.0, tximport package v1.12.3, DSSeq2 v1.24.0, ggtree, WGCNA, tidyverse v1.3.1, ggplot2 v3.3.5, vcfR v1.12.0, svgLite v2.0.0, fs v1.5.0, slider v0.2.2, tidyverse v1.0.4, treeio v1.16.1, ggtree v3.0.1, patchwork v1.1.1, gggenes v0.4.1, BWA-MEM2 v2.2.1, MultiQC v1.10, DeepVariant v1.1.0, GNU Parallel v20210422, GLNexus v1.3.1, Snakemake v6.5.1, bcftools v1.12, McScanX, Matlab, and SnipEff v4.3.11.

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The raw sequence data used for de novo whole genome assembly are available from the European Nucleotide Archive (ENA) under accession number PRJEB44810 (A. sativa cv. Sang) and from the Sequence Read Archive (SRA) under accession number PRJNA727490 (A. insularis BYU209) and PRJNA726919 (A. longiglumis CNS8138). Chromosome conformation capture (Hi-C) sequencing data are available from ENA under accession PRJEB43668 (A. sativa cv. Sang), PRJEB43670 (A. insularis BYU209) and PRJEB43669 (A. longiglumis CNS8138). Chromosome-scale sequence assemblies [pseudomolecules] are available from ENA under accession PRJEB44810 (A. sativa cv. Sang), PRJEB45088 (A. insularis BYU209) and PRJEB45087 (A. longiglumis CNS8138). The raw RNA-seq and WGS data generated in this study are available under ENA accession number PRJEB46365. Pseudomolecules, annotation data and analysis results are available in the Plant Genomics & Phenomics (PGP) Research Data Repository at http://dx.doi.org/10.5447/psp/2022/2. The DOI was registered using eDAL (https://edal.ipk-gatersleben.de/). Pseudomolecules, annotation data and associated analyses for A. sativa cv. Sang, A. longiglumis, and A. insularis are also available from GrainGenes60: Sang genome browser: https://wheat.pw.usda.gov/jw/?data=ggds/oat-sang; Sang data download: https://wheat.pw.usda.gov/GG3/content/avena-sang-download; A. longiglumis genome browser: https://wheat.pw.usda.gov/jw/?data=ggds/oat-longiglumis; A. longiglumis data download: https://wheat.pw.usda.gov/GG3/content/avena-longiglumis-download; A. insularis genome browser: https://wheat.pw.usda.gov/jw/?data=ggds/oat-insularis; A. insularis data download: https://wheat.pw.usda.gov/GG3/content/avena-insularis-download. The mass spectrometry proteomics data and ProteinPilot search result files have been deposited to MassIVE (UCSD, San Diego, CA, USA; https://massive.ucsd.edu) under accession number MSV000088727. The publicly available OT3098 oat genome data was generated by PepsiCo and Corteva Agriscience. This dataset (annotation version 2) has been obtained and is available from GrainGenes: https://wheat.pw.usda.gov/GG3/content/pepsico-oat-3098-hexaploid-oat-version-2-genome-assembly-release-collaboration-graingenes. Databases used in this study include PREFER release 19, Uniref download 2019-09-03, Pfam download 2019-09-03, Swiss-Prot, TAIR, TrEMBL, REdat_9.9_Psaceae section of the FGSB transposon library, Immune Epitope Database and Analysis Resource (https://www.iedb.org), PLACE and PlantCare promoter motif databases, and pfam2GO.

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Life sciences study design

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

Sample size
No statistical methods were used to establish sample size for genome sequencing and assembly. The two progenitor Avena accessions were chosen as the likely descendant of the hexaploid A, C and D subgenomes based on previous marker data analysis. The Sang cultivar was chosen as a representative Spring oat cultivar and to facilitate single gene mapping in a closely related TILLING population.

Data exclusions
All sequencing data generated and reported as raw data was used in the genome assembly and analyses.

Replication
In all analyses that support the genome assemblies, gene expression, proteomics, GC/MS and SEM, the number of replicates or iterations are indicated in materials and methods or supplemental tables. In each case, replications were successful and used. The genome assemblies themselves were validated using multiple methods i.e. BUSCO, genetic maps, HiC, and for A. sativa multiple comparisons to oat long-read assembly OT3098 were performed. This helped validate the other approaches.

Randomization
Randomization does not directly apply to the genome sequencing and assembly. However it does apply to some of the analyses conducted. In these cases, the group design and data pooling for computational analysis are described in the materials and methods and adhere to widely accepted standards. For example, bootstrapping was applied to all phylogenies computed (e.g. see Fig. 3a).

Blinding
Blinding does not apply to this study, as the study focuses on genome sequencing. This study focuses on plants genomics and the results of the study are not impacted by the concealment of treatment, data, or groups.

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