LETTER

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The genome of the recently domesticated crop plant sugar beet (*Beta vulgaris*)

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Sugar beet (*Beta vulgaris* ssp. *vulgaris*) is an important crop of temperate climates which provides nearly 30% of the world's annual sugar production and is a source for bioethanol and animal feed. The species belongs to the order of Caryophylalles, is diploid with $2n = 18$ chromosomes, has an estimated genome size of 714–758 megabases[1] and shares an ancient genome triplication with other eudicot plants[2]. Leafy beets have been cultivated since Roman times, but sugar beet is one of the most recently domesticated crops. It arose in the late eighteenth century when lines accumulating sugar in the storage root were selected from crosses made with chard and fodder beet[3]. Here we present a reference genome sequence for sugar beet as the first non-rosid, non-asterid eudicot genome, advancing comparative genomics and phylogenetic reconstructions. The genome sequence comprises 567 megabases, of which 85% could be assigned to chromosomes. The assembly covers a large proportion of the repetitive sequence content that was estimated[4] to be 63%. We predicted 27,421 protein-coding genes supported by transcript data and annotated them on the basis of sequence homology. Phylogenetic analyses provided evidence for the separation of Caryophyllales before the split of asterids and rosids, and revealed lineage-specific gene family expansions and losses. We sequenced spinach (*Spinacia oleracea*), another Caryophyllales species, and validated features that separate this clade from rosids and asterids. Intraspecific genomic variation was analysed based on the genome sequences of sea beet (*Beta vulgaris* ssp. *maritima*; progenitor of all beet crops) and four additional sugar beet accessions. We identified seven million variant positions in the reference genome, and also large regions of low variability, indicating artificial selection. The sugar beet genome sequence enables the identification of genes affecting agronomically relevant traits, supports molecular breeding and maximizes the plant's potential in energy biotechnology.

During the last 200 years of sugar beet breeding, the sugar content has increased from 8% to 18% in today's cultivars. Breeding has also actively selected for traits like resistance to viral and fungal diseases, improved taproot yield, monogermy of the seed and bolting resistance. After discovering a male sterile cytoplasm, breeders started to develop hybrid varieties and successfully increased yield[5]. Taxonomy assigns *Beta* to the Amaranthaceae family within Caryophylalles, an order comprising 11,510 species[6] including cacti, ice plants (Aizoaceae), other drought-tolerant species, and carnivorous plants such as pitcher plants (*Nepenthes*) and sundew (*Drosera*). Until now, no Caryophyllales species have been sequenced.

To provide an extended basis for comparative plant genomics and to support molecular breeding, we sequenced the double haploid sugar beet line KWS2320 as reference genotype, using the Roche/454, Illumina and Sanger sequencing platforms (Extended Data Table 1a, Supplementary Table 1). The initial assembly was integrated with genome-wide genetic and physical map information[2], resulting in 225 genetically anchored scaffolds (394.6 Mb), assigned to nine chromosomes (Table 1, Fig. 1, Extended Data Figs 1 and 2). The chromosomal nomenclature follows a previous study[7] describing a *Beta* karyotype at chromosome arm resolution. The genetically integrated assembly, 'RefBeet', comprised in total 569.0 Mb in 43,721 sequences (2,333 scaffolds and 41,388 unscaffolded contigs) and had an N50 size of 1.7 Mb with 77 scaffolds being of this size or larger. We incorporated Illumina sequencing reads generated from PCR-free libraries and analysed genotyping-by-sequencing data,

Table 1 | Assembly details by chromosome

Chromosome	Total size (Mb)		Number of sequences		N50 size (Mb)	Largest sequence (Mb)
	RefBeet	Incl. GBS data	RefBeet	Incl. GBS data		
1	41.5	51.6	12	28	6.46	8.26
2	39.5	50.4	23	38	2.63	9.20
3	32.3	40.4	17	25	3.06	5.16
4	31.1	53.4	27	56	1.34	4.51
5	56.2	65.4	37	54	2.31	8.59
6	57.8	65.6	31	45	3.38	6.74
7	50.9	54.7	28	42	2.47	10.43
8	40.1	48.0	21	33	2.86	7.39
9	45.2	50.2	29	43	2.29	8.58
	avg. 43.8 sum 394.6	avg. 53.3 sum 479.8	avg. 25 sum 225	avg. 40 sum 364	avg. 2.98	avg. 7.65
un	174.3	86.8	43,496	40,144	0.3	2.02

GBS, genotyping by sequencing.
un, unassigned fraction of the assembly.
avg., average.

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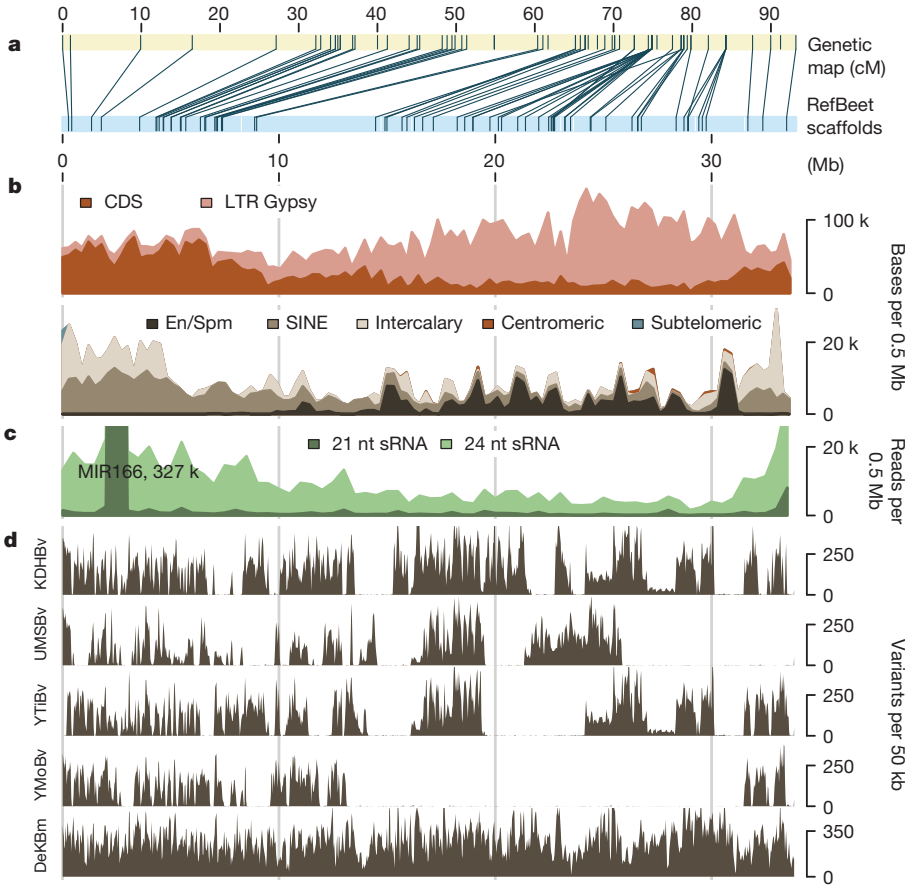

Figure 1 | Genomic features of RefBeet chromosome 1. For chromosomes 2–9 see Extended Data Figs 1 and 2. **a**, Positions of genetic markers in the genetic map[2] and the RefBeet assembly. **b**, Distribution of coding sequence (CDS) and repetitive sequence of the Gypsy type (LTR retrotransposons), the SINE type (non-LTR retrotransposons), the En/Spm type (DNA transposons), and three classes of satellite DNA (intercalary, centromeric, subtelomeric). **c**, Distribution of mapped small RNAs of 21 and 24 nucleotides (nt). The large peak of 21 nt reads (about 327,000 reads mapped) corresponds to the highly expressed microRNA MIR166. **d**, Distribution of genomic variants in four sugar beet accessions and sea beet (DeKBm) compared to RefBeet. Shared and individual low-variation regions per accession are visible (for example, region 30–31 Mb is shared among the sugar beet accessions KDHBv, UMSBv, YTiBv, YMoBv).

leading to an optimized assembly of 566.6 Mb in 2,171 scaffolds and 38,337 unscaffolded contigs. The N50 size was 2.01 Mb (the 72nd scaffold) and the chromosomally assigned fraction 84.7% (Table 1). The assembled part of the genome is assumed to represent the unique regions as well as repetitive regions, which are either short enough to be placed in a unique sequence context or divergent enough to behave as unique entities. A k-mer analysis of Illumina data indicated a genome size of 731 Mb (Extended Data Fig. 3a). We located 94% of publicly available isogenic expressed sequence tags (ESTs) in RefBeet, suggesting that gene-containing regions are comprehensively covered. A sequenced bacterial artificial chromosome (BAC) clone[8] was compared to the corresponding region in RefBeet and found to be correctly assembled within one scaffold. On average, one mismatch and one insertion or deletion (indel) error occurred in 10 kb. RefBeet resolved regions of recombination suppression in centromeric and pericentromeric regions of chromosomes, flanked by regions showing enhanced recombination rates (Extended Data Fig. 4).

We identified 252 Mb (42.3%) of RefBeet as repetitive sequence (Supplementary Data 1). The largest group was long terminal repeat (LTR) retrotransposons (Extended Data Fig. 5a). Gypsy-like elements were enriched in centromeric and pericentromeric regions (Fig. 1, Extended Data Figs 1 and 2). Non-LTR retrotransposons of the long interspersed nuclear element (LINE) type were dispersed, whereas short interspersed nuclear elements (SINEs) were enriched towards chromosome ends. Three major satellite classes were organized in large arrays (Fig. 2). By analysing unassembled genomic data we estimated total amounts of 15.4 Mb centromeric, 6.0 Mb intercalary, and 0.6 Mb subtelomeric satellite DNA, as well as 10.0 Mb of 18S-5.8S-25S and 5S ribosomal genes.

A total of 27,421 protein-coding genes supported by mRNA evidence (Supplementary Table 2) were predicted in RefBeet; 91% included start and stop codons (Supplementary Table 3). The majority of the genes (73.6%) were found within chromosomally assigned scaffolds with on average 5.2 genes per 100 kb, a gene length of 5,252 bp including introns, a coding sequence length of 1,159 bp and 4.9 coding exons per gene. The codon usage was similar to other dicot species (Supplementary Table 4). Homology-based annotation of non-coding RNA genes resulted in 3,005 predictions of tRNAs, microRNAs, small nucleolar RNAs, spliceosomal RNAs and ribosomal RNAs, mainly supported by evidence from isogenic small RNA data (Extended Data Fig. 5b–e, Extended Data Table 1b, Supplementary Table 5).

Based on the translated *Beta vulgaris* gene set and the protein sets of nine other plants (Extended Data Table 1d) we determined 19,747 phylogenetic trees, collectively called 'phylome'[9], and inferred orthologous and paralogous gene relationships (Extended Data Fig. 6a). Previous studies left the phylogeny of rosids, asterids and Caryophyllales unresolved[10] or classified Caryophyllales as a subclade of asterids[11]. A species tree inferred from the collection of gene trees strongly suggested that *Beta vulgaris* branched off before the separation of asterids and rosids (Fig. 3). Thus, according to our data, Caryophyllales represent the most basal eudicot clade among the studied species. The fraction of species-specific genes within eudicots (Fig. 3) was the largest for sugar beet, reflecting its phylogenetic position. The analysis of paralogous genes provided evidence for the absence of a lineage-specific whole genome duplication in *Beta vulgaris* supporting previous studies[2] (Extended Data Fig. 6b–e, Supplementary Table 6).

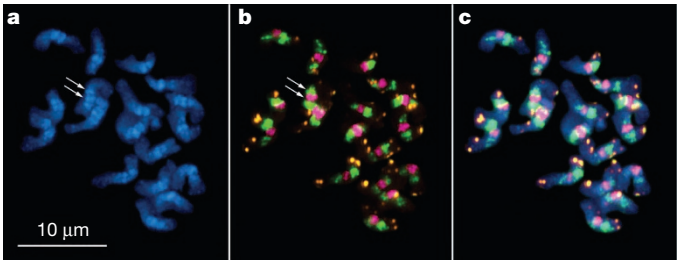

Figure 2 | Fluorescent *in situ* hybridization (FISH) analyses of *Beta vulgaris* chromosomes at early metaphase. **a**, Chromosomes were stained with 4′,6-diamidino-2-phenylindole (DAPI, blue); large blocks of heterochromatin are visible (arrows). **b**, *In situ* hybridization using the major satellites pBV (centromeric, red), pEV (intercalary, green) and pAV (subtelomeric, orange). **c**, Overlayed images of **a** and **b** show the coverage of chromosomes by satellite DNA. Scale bar, 10 µm.

We functionally annotated 17,151 RefBeet genes (63%) based on sequence homology (Supplementary Data 2). The number of disease resistance genes detected was comparatively small, particularly for the STK-domain containing classes (Supplementary Table 7, Supplementary Data 3). In contrast to previous studies[12,13], we found a TNL class resistance gene in the genomes of sugar beet (Bv_22240_ksro) and spinach, both belonging to Amaranthaceae. The phylome tree of Bv_22240_ksro indicated that the presence of a single TNL class gene is a feature of Amaranthaceae, whereas expansion of this gene family is typical for rosids and asterids. The functional categories of expanded and potentially lost gene families (Extended Data Fig. 5f, Supplementary Tables 8, 9) indicate that genes involved in defence and stress compensation represent vital evolutionary targets. The number of transcription factors identified in RefBeet was the lowest of all species studied (Supplementary Table 10). The reference genome sequence enables future experimental approaches to determine if lower gene numbers may alter transcriptional network topologies; Caryophyllales may harbour unknown genes involved in transcriptional control. We identified four sucrose transporter (SUT) orthologues in RefBeet. Phylogenetic analysis including known sucrose transporters suggested a duplication of the *SUT1* gene in Amaranthaceae followed by extensive mutation of one paralogue (Extended Data Fig. 7a). The genome sequences of sugar beet and spinach, both containing the four SUT genes, are an excellent basis for studying the implications of this duplication event.

Previous studies addressing the variation within the genus *Beta* indicated high divergence between genotypes[2,14]. We generated genome sequences of four non-reference sugar beet double haploid accessions (KDHBv, UMSBv, YMoBv, YTiBv) and characterized the genome-wide variation (Extended Data Table 1a, c, Supplementary Tables 3, 11–13). Within RefBeet we identified 7.0 million positions which were variant (77% substituted, 23% deleted) in at least one of the other accessions and 274.9 million positions which were unchanged in all five accessions. We found 2.9 million variants on average per non-reference accession. Coding regions had a prevalence of indels of length three or multiples of three (44%), compared to non-coding regions (16%). The distribution of variants revealed large regions of low variation (Fig. 1, Extended Data Figs 1, 2, 8a–c). Such variation 'deserts' were found in all chromosomes and in all accessions, which might reflect extensive cross-breeding with a limited number of haplotypes in the breeding material, a founder effect, or a bottleneck at the establishment of the crop. However, most of the variation deserts were accession-specific (Extended Data Fig. 8d), probably owing to recombination events that have occurred since the introduction of founder haplotypes into breeding lines. The four accessions shared 50.6 Mb of variation deserts along RefBeet containing 1,824 predicted RefBeet genes (Gene Ontology (GO) term enrichment see Supplementary Table 14). Genes in these regions, analysed in 24 additional sugar beet accessions, showed higher sequence conservation (Extended Data Fig. 8e). These findings suggest that regions of low variation are not maintained by chance, but are rather the result of breeders' selection towards certain genes contained in those regions. The sea beet *Beta maritima* is fully interbreedable with sugar beet and commonly used as a valuable source of resistances against biotic or abiotic stress[15]. We sequenced its genome and identified a total of 75 Mb as variation desert, of which 67 Mb were shared with at least one of the four non-reference *Beta vulgaris* accessions. These regions may represent traces of breeding activities which aimed at introducing sea beet traits into sugar beet. The gene *BvBTC1*, encoded by the *B*-locus[16] and located in a 1.1 Mb RefBeet scaffold on chromosome 2, plays an important role during vernalization. Cultivated lines are homozygotes for the *B* allele resulting in a biennial life cycle. The *B*-locus is located in variation deserts of all five sugar beet lines, whereas the genome of the annual wild form *Beta maritima* shows high variation at this locus, demonstrating that breeding has shaped the genome of sugar beet.

Sugar beet is a hybrid crop based on seed pool lines (male steriles, monogerms) and pollen pool lines (pollinators, multigerms). We identified regions of potentially fixed differences between the two groups: the

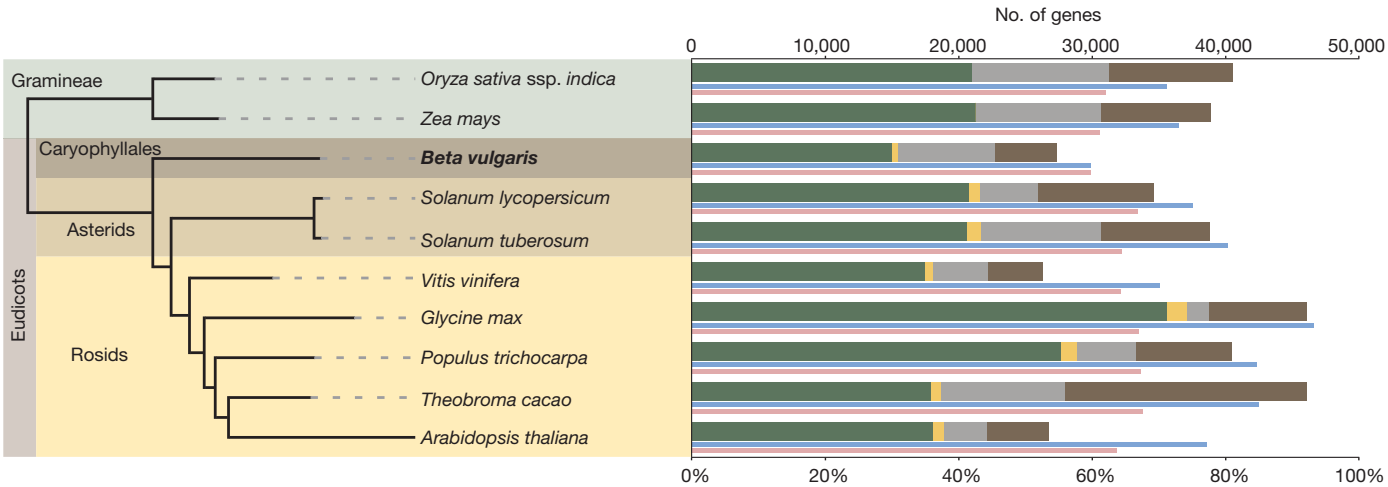

Figure 3 | Phylogenetic relationship of 10 sequenced plant species and comparative gene analysis. Species tree based on maximum-likelihood analysis of a concatenated alignment of 110 widespread single-copy protein sequences (left). The upper bar per species (right with scale on the top) indicates the number of widespread genes that are found in at least 9 of the 10 species (green); eudicot-specific genes that are found in at least 7 of the 8 eudicot species (yellow); species-specific genes with no homologues in other species of this tree (light grey); and remaining genes (brown). The slim bars per species (scale on the bottom) represent the percentage of genes with at least one paralogue (blue) and the percentage of sugar beet genes that have homologues in a given species (pink), respectively.

intersection of shared low-variation regions in seed pool lines and shared high-variation regions of identical variation patterns in pollen pool lines comprised 311 genomic regions (1.6 Mb in total) containing 119 genes.

We performed evidence-based gene predictions in the assemblies of KDHBv, UMSBv, YMoBv and YTiBv. Based on the comparison of 2,112 single copy genes, UMSBv had the largest genetic distance to RefBeet (Extended Data Fig. 7b). The number of accession-specific genes ranged from 79 (RefBeet) to 271 (UMSBv). Genes were analysed for the ratio of non-synonymous to synonymous substitutions, altered start and stop sites, new stop codons, modified splice donor or splice acceptor sites and indels, revealing extensive variation in coding regions (Supplementary Tables 15, 16 and Extended Data Fig. 8f). In addition to allelic variation, the variation in gene content may contribute to heterosis, as has been suggested for maize[17].

The availability of the sugar beet genome sequence very much simplifies fine-mapping of quantitative trait loci and the discovery of causal genes, as single-nucleotide polymorphism (SNP)-based markers can be designed for any region of the genome. Association mapping to identify regions of shared ancestry in sugar beet lines requires at least 100,000 variant positions for genotyping. Such positions can now be selected from a catalogue of seven million variants. The genome sequence facilitates further experimentation to characterize gene functions, which accelerates the identification of rewarding targets for transgenic manipulation, and represents an important foundation for molecular and comparative studies in sugar beet, Caryophyllales and flowering plants. The data presented are key to improvements of the sugar beet crop with respect to yield and quality and towards its application as a sustainable energy crop.

METHODS SUMMARY

Genome sequencing and assembly. Genomic DNA isolated from root and leaf material was sequenced on the Roche/454 FLX, Illumina HiSeq2000 and ABI3730 XL sequencing platforms. The Newbler software was applied on 454, Illumina and Sanger sequencing data to assemble the reference genotype (RefBeet). Contigs of putative bacterial origin and those smaller than 500 bp were removed. Additional lines were sequenced on the HiSeq2000 platform and were assembled using SOAPdenovo. We performed gap-closing and homopolymer error correction using Illumina reads from PCR-based and PCR-free libraries (Extended Data Fig. 3b, c). Chromosome-wise scaffolding using genetic and physical mapping data was assisted by SSPACE (Methods and Supplementary Methods).

Gene annotation. Prediction of protein coding genes was performed using the AUGUSTUS pipeline, with Illumina mRNA-seq reads and other cDNA read data as supporting evidence. Gene models were filtered for transposable element homology. Small and other non-coding RNAs were identified based on homology searches and based on Illumina sequencing data. Repeats were predicted using RepeatModeler, followed by manual curation of the predictions (Methods and Supplementary Methods).

Intraspecific variation. Variant positions (substitutions, indels) were identified by read mapping and scaffold alignment (Methods and Supplementary Methods).

Phylogenetic analysis and species tree reconstruction. The longest protein sequence of each RefBeet gene was used for a Smith–Waterman search against the protein sets of nine other plant species. Alignments were generated and quality-filtered, and phylogenetic trees were calculated for each *Beta vulgaris* sequence. A species tree was generated from a super-tree of all trees and by multi-gene phylogenetic analysis of high-confidence 1:1 orthologues.

Functional annotation. Protein coding gene predictions were functionally annotated based on protein signatures and orthology relationships.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions H.H., B.W. and J.C.D. conceived the study, H.H., D.H., T.R.S., R.R. and B.W. prepared sequencing data, J.C.D., A.E.M., D.H., S.C.-G., F.Z., H.T., O.R., R.S., A.G., B.S., T.K., P.F.S., T.S. and T.G. designed experiments and analysed the data, H.L. participated in project design, J.C.D., H.H. and A.E.M. wrote the paper with input from all other authors. All authors have read and have approved the manuscript.

Author Information Sequencing raw data (genomic and transcript sequences) have been submitted to the SRA archive with the study accession number SRP023136. The NCBI Bioproject accession is PRJNA41497. The whole-genome shotgun assemblies have been deposited at DDBJ/EMBL/GenBank under the accessions AYZS00000000–AYZY00000000. The GenBank accession numbers KG026656–KG039419 were assigned to BAC end sequences and JY274675 –JY473858 to fosmid end sequences generated in this study. Plant material for *Beta vulgaris* genotype KWS2320 and *Beta maritima* 9W_2101 (DeKBm) are available as seeds by signing a material transfer agreement (MTA). Reprints and permissions information is available at www.nature.com/reprints. A sugar beet website including a genome browser has been set up at http://bvseq.molgen.mpg.de, providing access to assemblies, annotations, gene models and variation data. The sugar beet phylome can be accessed at http://phylomeDB.org. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to H.H. (Heinz.himmelbauer@crg.es) or B.W. (bernd.weisshaar@uni-bielefeld.de).

METHODS

Sequencing and assembly. Genomic DNA isolated from root and leaf material was sequenced on the Roche/454 FLX, Illumina HiSeq2000 and ABI3730 XL sequencing platforms. The plant material included five double haploid and two inbred sugar beet breeding lines (*Beta vulgaris* ssp. *vulgaris*; referred to as *Beta vulgaris*), one wild beet accession (*Beta vulgaris* ssp. *maritima*; referred to as *Beta maritima*), and one spinach accession (*Spinacia oleracea*). Additionally, 15 genotypes from an F2 panel of a *Beta vulgaris* cross used to generate beet genetic maps[2,14] were sequenced at low coverage (Supplementary Methods).

Illumina genomic sequencing was performed on a HiSeq2000 sequencing instrument with 2 × 100 nt for paired-end reads and 2 × 50 nt for mate-pair reads (Supplementary Tables 1 and 11). Roche/454 genomic single-read and mate-pair sequencing was performed on a Roche/FLX sequencing instrument using Titanium XLR70 sequencing kits (Roche/454 Life Sciences). End-sequencing of genomic BAC and fosmid libraries introduced previously[2,18,19] was performed on an ABI3730 XL DNA Analyzer. Genomic Roche/454 and Illumina data, BAC ends and fosmid ends were filtered for low-quality sequence, contamination and redundancy, and all data sets were assembled together on a 512 GB access-random memory (RAM) computer using the Newbler software (v2.6 20110630_1301, parameters -fe exclude_list -siod -nrm -scaffold -large -ace -ar -a 40 -l 500 -cpu 48).

We removed potential bacterial contigs and scaffolds based on three criteria: a GC content of 60% or higher, the presence of predicted genes without sugar beet cDNA support, and the absence of both sugar beet repeats and genes supported by cDNA data. The assembly size was determined by adding up the lengths of the scaffolds and the lengths of additional unscaffolded contigs larger than 500 bp (smaller contigs were removed). The N50 size refers to this assembly size as 100% and reports the length of the scaffold or contig that spans the 50% mark after sorting the sequences by length.

Illumina-only assemblies were performed on 100 GB and 256 GB RAM computers using the SOAPdenovo[20] software v1.05 (SOAPdenovo-63mer, -K 49, pair_num_cutoff = 3, map_len = 32) followed by gap filling using GapCloser v1.12.

mRNA-seq sequencing was carried out on the Illumina Genome Analyzer (GA) IIx and Illumina HiSeq2000 sequencing instruments. From each library, one lane of data was generated with read lengths of 2 × 54 nt on the GA and 2 × 50 nt on the HiSeq2000. Small RNA libraries were sequenced with 36 nt reads on the GA and 50 nt reads on the HiSeq2000. Additional cDNA sequences from sugar beet were generated by Roche/454 Life Sciences on the GS20 platform with an average length of 106 bp. The transcript data generated are summarized in Supplementary Table 2.

Integration with genetic and physical maps as well as genotyping-by-sequencing (GBS) data. Sequence information of anchored markers in genetic and physical maps[2] was used to assign scaffolds to chromosomes and to build connections between scaffolds. Confirmation and further genetic integration was derived from generating and analysing GBS data (see Supplementary Methods). To establish new connections between scaffolds, a group of scaffolds placed as neighbours based on genetic integration was used as input for SSPACE[21] (v1.1, parameters -x 0 -k 1) together with the six largest paired data sets (Illumina 6 kb, Roche/454 7 kb, 10 kb and 20 kb, fosmid ends, BAC ends). The output was manually corrected if necessary. Analyses and control steps were coded in Perl v5.8.9 or used UNIX shell commands.

Correction of small indels in the assembly and gap closing. For consensus sequence correction we mapped quality filtered Illumina reads (2 × 100 nt, 93-fold genome coverage) against the assembly using BWA[22] v0.5.9 allowing for 3 edits. Indels were identified using SAMtools mpileup[23] v0.1.18. A total of 9,101 bp insertions and 60,685 bp deletions were corrected in the consensus sequence. Indel errors were corrected if error positions were covered by at least 10 reads, if at least 60% of them showed the same indel and if the indel was confirmed on both strands. The criteria were validated using the Sanger sequence of the sugar beet BAC insert ZR47B15 (ref. 8).

Gap closing was performed using 670 million Illumina paired-end reads generated from two PCR-amplified libraries with insert sizes 600 nt and 250 nt, and from five PCR-free libraries with insert sizes 200–700 nt as input for GapCloser[20] (v1.12-r6, default parameters and -p set to 31).

Annotation of repetitive elements. The *de novo* identification and classification of repeats within the RefBeet assembly was performed using RepeatModeler (http://www.repeatmasker.org). RepeatModeler v1.0.5 was installed along with RECON[24] v1.07, RepeatMasker 'open 3-3-0', and RMBlast v1.2 with BLAST v2.2.23 (all loaded from http://www.repeatmasker.org), RepeatScout[25] v1.0.5 (http://bix.ucsd.edu/repeatscout/), Tandem Repeat Finder[26] (trf404 loaded from http://tandem.bu.edu/), and the RepeatMasker libraries (http://www.girinst.org/server/RepBase/) as of September 2011. RepeatModeler was applied on the database file created by the BuildDatabase subprogram which was run on the RefBeet assembly. The output was used as library for RepeatMasker (parameters -e crossmatch -pa 20 -gff) to generate a masked version of the assembly and to get the genomic positions of the repeat annotation. The repetitive fraction of the assembly was determined based on the RepeatMasker output. The automated repeat classification provided by RepeatModeler was refined by manual curation of the data (see Supplementary Methods). The repeat families along with the combined automated and manual classification are listed in Supplementary Data 1. The fractions of different repeat classes annotated in RefBeet are shown in Extended Data Fig. 5a.

The distribution of small RNAs (Fig. 1 and Extended Data Figs 1 and 2) was analysed by mapping 677.8 million adaptor-trimmed small RNA sequences of three libraries (see Supplementary Table 5) against RefBeet using BWA v0.6.1. Reads shorter than 15 bases after trimming were removed. The mapping seed length was set to 15. If a read mapped to multiple locations one random location was kept. Custom Perl scripts were used to locate mapped reads within the annotation of non-coding RNAs. The read length distribution and the chromosome-wide distribution of mapped reads were computed with Perl and plotted with R v2.15.

Coding gene prediction. The evidence-based *de novo* annotation of coding genes was performed applying the program AUGUSTUS[27] v2.5.5 on the RefBeet assembly. The evidence was provided by 616.3 million filtered Illumina mRNA-seq reads (mainly generated as paired-ends) from five *Beta vulgaris* accessions and different tissues, 282,169 cDNA single-end reads generated on a Roche/454 GS20 platform, and 35,523 EST sequences from public databases. The Roche/454 reads and most of the ESTs were derived from genotype KWS2320. AUGUSTUS settings were: using *Arabidopsis* training data, reporting untranslated regions in addition to the coding sequences, reporting alternative transcripts if suggested by hints, and accepting introns that start with AT and end with AC in addition to introns with starts flanked by GT-AG and GC-AG.

For each *Beta vulgaris* accession we initially predicted 30,339 to 36,589 genes. After removal of (retro)transposon gene candidates, the gene sets used for downstream analyses consisted of 25,368 to 31,355 genes (Supplementary Table 3). Of those genes, 77–94% had both start and stop codon, and the fraction of predictions completely supported by cDNA was 48–61%.

We identified transposable element-related genes in the automated gene prediction of RefBeet by screening the phylomes for GO terms specific to transposable elements, by running the program 'TransposonPSI' (http://transposonpsi.sourceforge.net/), and by analysing the genomic positions of the repeat annotation (Supplementary Methods). In total, 4,643 transposable element candidates were removed from the initial set of 32,064 evidence-based genes predicted in RefBeet by AUGUSTUS.

The predicted genes of assemblies generated with SOAPdenovo (Supplementary Tables 3, 11) were screened for overlap with sequences of transposable elements contained in the repeat annotation. The assemblies were masked using RepeatMasker, and gene predictions were omitted from further analyses if at least one base of their coding parts overlapped with an annotated transposable element.

Plant species analysed in comparative studies. Comparative analyses were carried out based on data from seven dicot (five rosids and two asterids) and two monocot species: *Arabidopsis thaliana*, *Glycine max*, *Populus trichocarpa*, *Theobroma cacao*, *Vitis vinifera*, *Solanum lycopersicum*, *Solanum tuberosum*, *Oryza sativa* ssp. *indica* and *Zea mays* (Extended Data Table 1d, Supplementary Methods).

Annotation of non-coding RNA genes. Non-coding RNA genes were predicted using the programs tRNAscan-SE[28], RNammer[29], and BLAST[30], and based on database searches in Rfam[31], the plant snoRNA database[32], GenBank[33], and the ASRG database[34]. To support the predictions, we mapped 677.8 million Illumina small RNA reads generated from root, inflorescence and leaf material of the reference genotype against RefBeet (Supplementary Table 5). Reads were adaptor trimmed (custom Perl script) and mapped using BWA v0.6.1 with a seed length of 15 bases and one edit allowed in the alignment. The cDNA coverage was determined with SAMtools mpileup and custom Perl scripts.

Phylome reconstruction and orthology/paralogy predictions. The longest protein sequence for each gene annotated in RefBeet was used for a Smith-Waterman search (E-value cutoff 1×10^{-5}, matching length >50% of the query sequence) against the protein sets of nine other species. Alignments were generated and quality-filtered, and phylogenetic trees were calculated for each *Beta vulgaris* sequence (see Supplementary Methods). The collection of phylogenetic trees is referred to as the sugar beet 'phylome', based on which we inferred the orthologous and paralogous relationships of the genes by considering each node as either a speciation or duplication event.

Species tree reconstruction. A phylogeny describing the evolutionary relationships of the species included in the phylome was inferred using two complementary approaches resulting in identical tree topologies (Fig. 3). First, a super-tree was inferred from all the trees in the phylome (19,747 trees) by using a gene tree parsimony approach as implemented in the DupTree algorithm[35]. This approach is different from other super-tree approaches (such as finding the majority-rule consensus) as it finds the species topology with the minimum total number of

duplications implied when reconciling a collection of gene family trees (that is, the phylome) with that species topology. Second, 110 gene families with high-confidence one-to-one orthology in at least 9 of the 10 species were used to perform a multi-gene phylogenetic analysis. Protein sequence alignments were performed as described (see phylome reconstruction in Supplementary Methods) and concatenated into a single alignment. Species relationships were inferred from this alignment using a maximum likelihood (ML) approach as implemented in PhyML[36] using the Jones–Taylor–Thornton (JTT) evolutionary model; for 97 of 110 gene families this model was best-fitting. Branch supports were computed using an aLRT (approximate likelihood ratio test) parametric test based on a chi-square distribution. Both complementary approaches resulted in an identical topology. Such congruence is suggestive that a correct phylogeny was found.

To track specific or shared genes in the species tree an all-against-all BLAST search of the protein sets of the ten species was performed (E-value cutoff 1×10^{-5}). The patterns of homology across species and clades were computed. The result was categorized as widespread genes, eudicot-specific genes and species-specific genes (Fig. 3).

Whole genome duplication analysis using collinear blocks of coding genes. We performed a *Ks* analysis of 370 paralogous gene pairs forming 34 collinear blocks (Extended Data Fig. 6c–e). Collinear blocks of coding genes were determined using MCScanX[37] applied on the RefBeet protein set (longest protein isoform per gene). Protein sequences were aligned against themselves using BLASTp, the top 5 alignments per gene were kept. High-confidence collinear blocks with an E-value lower than $1 \times 10^{-10}$and a score larger than 300 were selected (parameters suggested by MCScanX). A total of 34 blocks of 7–35 gene pairs (on average 11 pairs, in total 370 pairs) were found. *Ks* values were calculated using MCScanX, which implements the Nei-Gojobori algorithm[38].

Functional annotation of protein coding genes. Protein coding gene predictions were functionally annotated based on protein signatures and orthology relationships. In the protein signature approach, each sugar beet protein was inspected for different signatures such as families, regions, domains, repeats, and binding sites using InterProScan[39] v4.8 and a set of different databases (PROSITE, PRINTS, Pfam, ProDom, SMART, TIGRFAMs, PIR superfamily, SUPERFAMILY, Gene3D, PANTHER, HAMAP). Additionally, BLAST searches against SwissProt[40], KEGG[41], and KOG[42] databases were performed, and annotations were extracted. In the phylogeny-based approach 15,263 one-to-one orthology relationships between *Beta vulgaris* genes and GO-annotated genes of other plant species were inferred from trees in the sugar beet phylome. The annotations from all sources were combined, and a merged annotation table was generated (Supplementary Data 2).

We predicted and classified resistance gene analogue (RGA) genes by applying a modified version of an HMM-based pipeline[43] (Supplementary Methods). The numbers of RGAs detected in *Beta vulgaris* and nine other plant species are listed in Supplementary Table 7. The gene identifiers of all putative RGAs in the *Beta vulgaris* genome are listed in Supplementary Data 3. Proteins were classified into 56 transcription factor families and subfamilies based on protein domains defined by InterPro motives[44] indicating DNA binding or other domains characteristic to transcription factors[45] (see Supplementary Methods). The numbers of transcription factors identified per species and per transcription factor class are listed in Supplementary Table 10. Sucrose transporter (SUT) proteins were identified by comparison of 40 known SUT protein sequences of 15 higher plants[46] against RefBeet (see Supplementary Methods).

Expanded and potentially lost gene families in *Beta vulgaris*. Expanded gene families were detected by searching the *Beta vulgaris* phylome for genes specifically duplicated in *Beta vulgaris*. To determine potentially lost gene families in *Beta vulgaris* the set of protein sequences of *Arabidopsis* was used for a BLAST search (E-value cutoff 1×10^{-5}, minimum 50% length of the query protein) against *Beta vulgaris* proteins.

Intraspecific variation. Four non-reference sugar beet accessions were sequenced (KDHBv, UMSBv, YMoBv, YTiBv). Additional data for the reference accession, processed in the same way, was generated as a control and quality measure (referred to as 'RefBv'). We generated a merged variant collection from RefBeet positions covered by read mapping or scaffold alignment and distinguished positions that were identical, variant (substituted or deleted), or uncalled ('N' in either RefBeet or in the other accession's assembly). The number of insertions, deletions, substitutions and mixed events (indel plus substitution) was counted.

Substitutions, insertions and deletions contained in coding regions were extracted, counted and categorized using a custom Perl script. Categories were indels, synonymous and non-synonymous codon alterations, changed start and stop codons, new stop codons, and splice donor or acceptor sites alterations (Supplementary Table 15). Splice sites were considered as altered if variants affected the first two or the last two bases of an intron. The standard genetic code was used to translate the coding sequence into amino acids and stop codons. The number of transitions (A↔G, T↔C), transversions (A↔T, C↔G, A↔C, G↔T), and indels of length three or multiples of three (Extended Data Fig. 8f) were determined using a custom Perl script.

We discovered regions in the genome with low variant rates (≤2 variants per 2 kb window) which we refer to as variation deserts (Extended Data Fig. 8a–d). Variant positions, identical positions and excluded positions (mainly due to low coverage) were counted per 2 kb intervals (shifted by 1 kb). An interval was considered as desert interval if at most two variants and at most 500 excluded bases were contained. A variant desert was defined as stretch of adjacent desert intervals. Genes located within a variant desert with at least 90% of the genomic length (CDS and UTRs) were considered as variation desert genes. We determined the frequency of each GO term assigned to the group of 1,824 desert genes and the remaining 25,597 genes. GO terms were kept if they were at least ten times more frequent within the group of desert genes (that is, GO terms with odds ratio <10 were removed). The probability that the enrichment occurred by chance was calculated using the two-tailed Fisher exact test (*P* value cutoff 0.05, no correction for multiple testing). Enriched GO terms are listed in Supplementary Table 14. The conservation of 51 RefBeet genes inside and outside of shared variation deserts was measured by screening for polymorphisms within an extended panel of 24 sugar beet genotypes representing different breeding programs (Supplementary Methods).

To analyse the presence or absence of RefBeet genes within the genomes of other double haploid accessions, Illumina paired-end reads of KDHBv, UMSBv, YTiBv, and YMoBv were mapped against the RefBeet assembly using BWA v0.5.9 (3 edits allowed). Only uniquely mapping reads were considered. Before inferring absence or presence of a gene in the non-reference accessions, the coverage of RefBeet genes was confirmed by mapping Illumina data of the reference genotype: a CDS part of RefBeet genes was ignored if less than 90% of its length was covered by RefBv reads (10.6% of 27,421 RefBeet genes entirely ignored). Genes were considered absent in one of the other accessions if less than 1% within the total of retained CDS length was matched by reads from the non-reference accession. To detect accession-specific genes, the procedure was performed for each accession separately.

The phylogenetic tree of the sugar beet accessions was constructed based on a set of 2,112 single copy genes shared between the five sugar beet accessions. The protein sequences were used to generate multiple alignments based on which a phylogenetic tree was constructed (Extended Data Fig. 7b).

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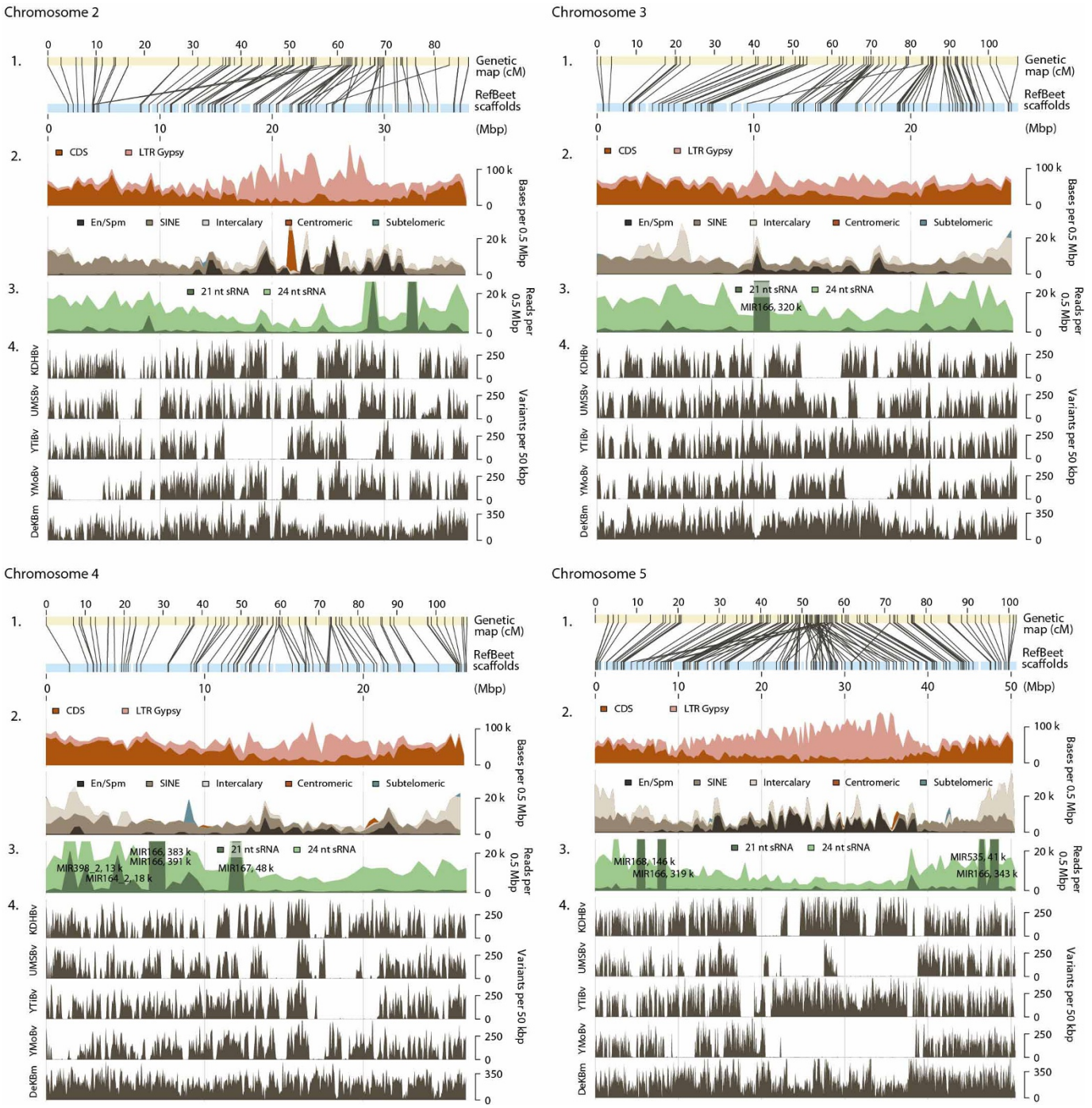
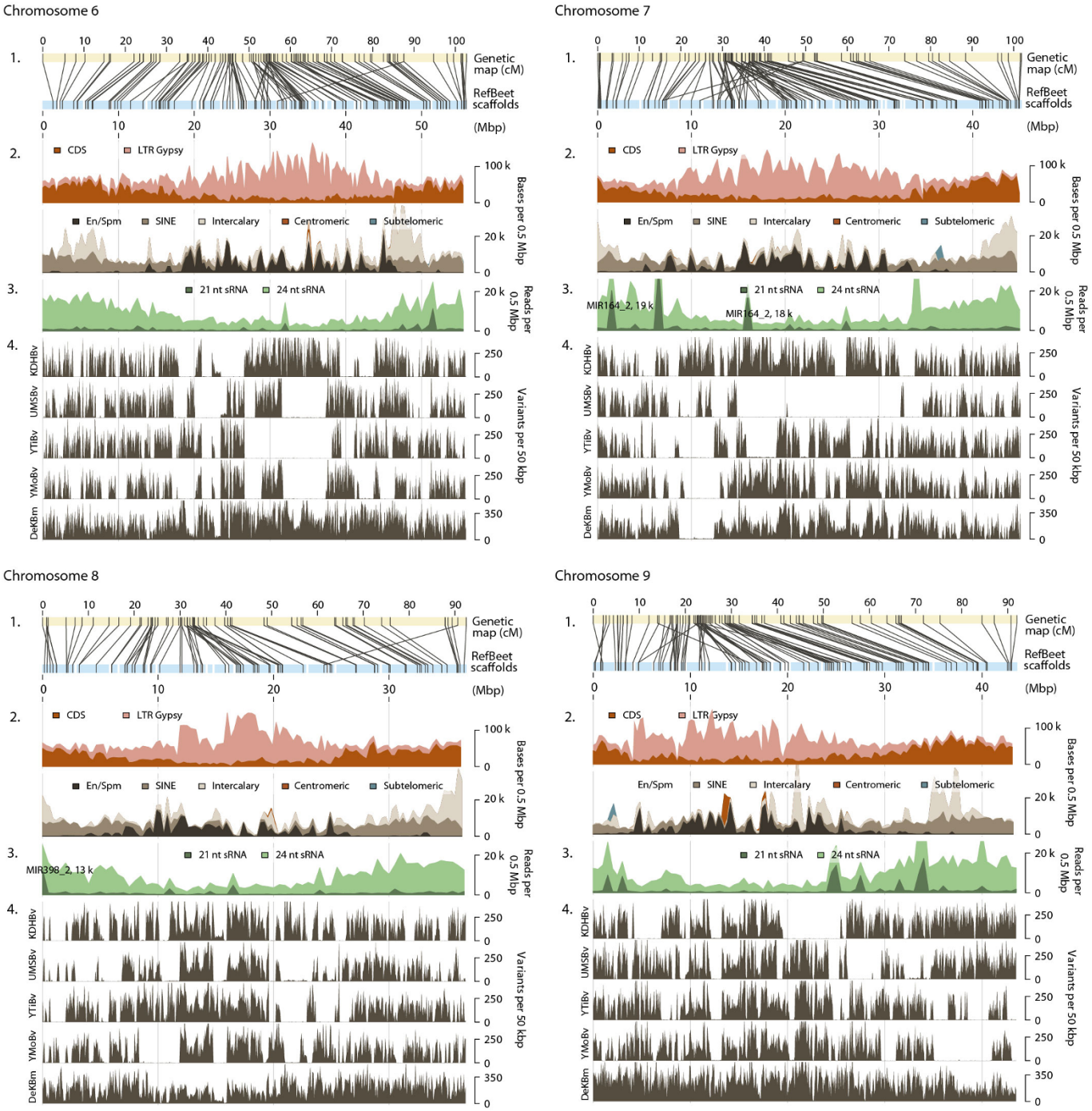
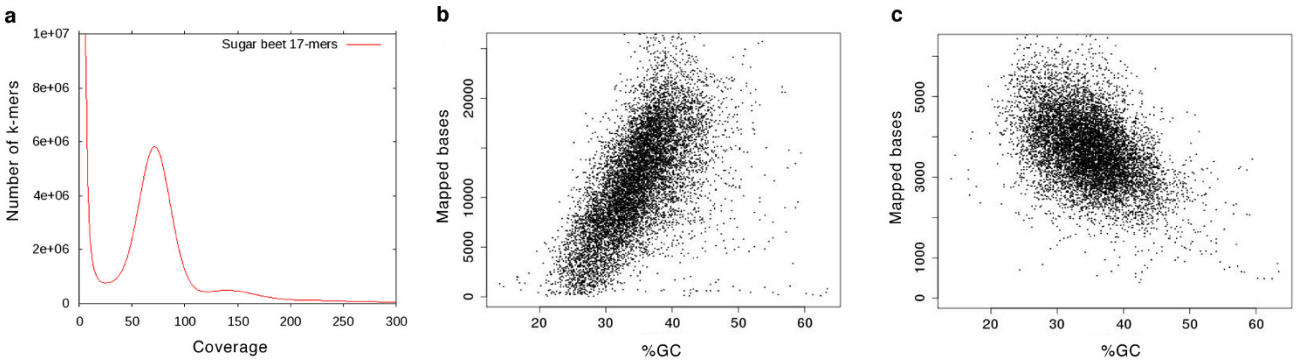


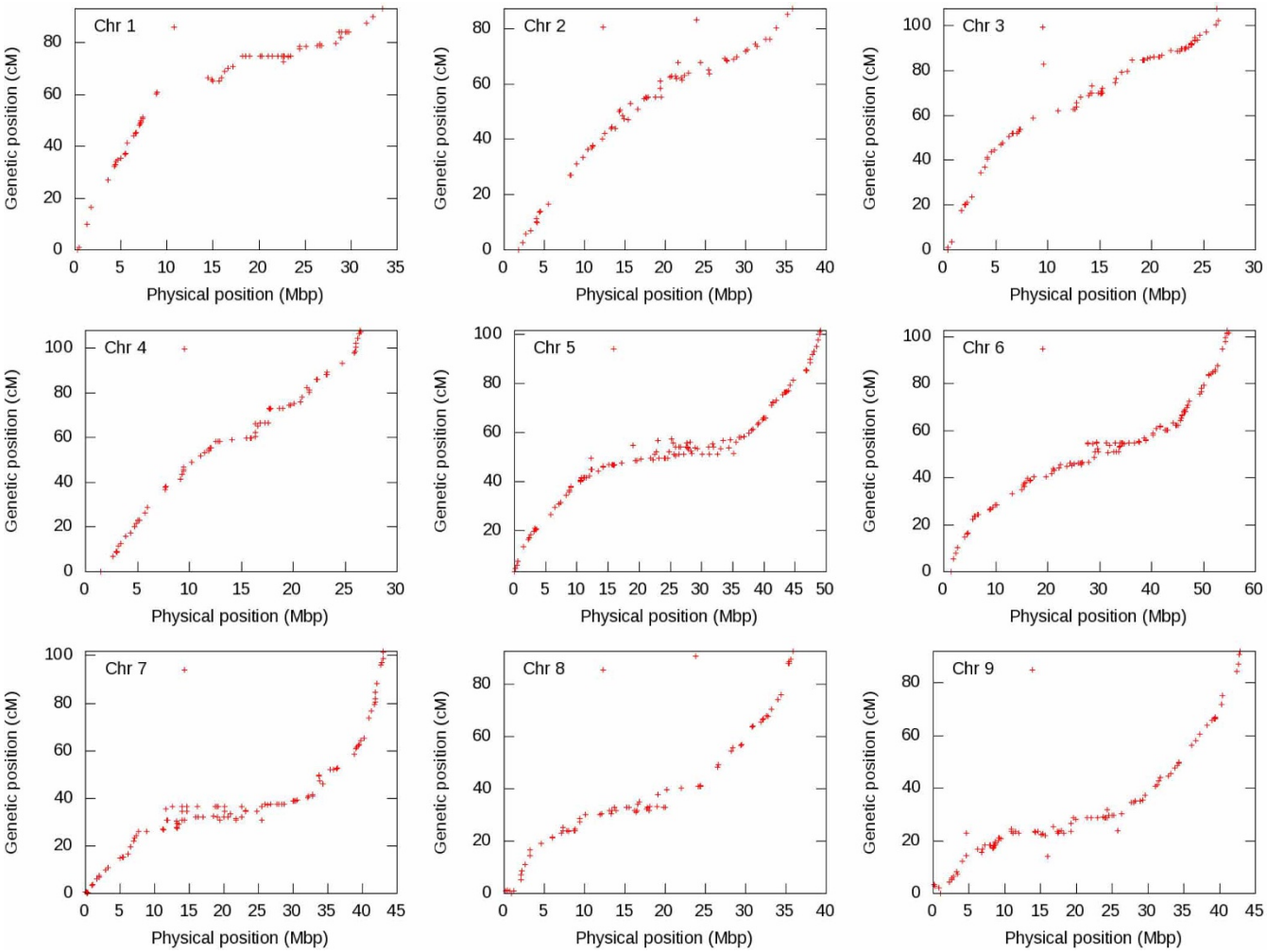
Extended Data Figure 1 | Genomic features of RefBeet chromosomes 2–5. Section 1 shows the positions of genetic markes in the genetic map[2] and RefBeet. Section 2 shows the distribution (stacked area graph) of predicted coding sequence (CDS) and repetitive sequence of the Gypsy type (LTR retrotransposon), the SINE type (non-LTR retrotransposon), the En/Spm type (DNA transposon), and three classes of satellite DNA (intercalary, centromeric, subtelomeric). The number of bases per feature is displayed in windows of 500 kb (shifted by 300 kb). Section 3 shows the distribution (stacked area graphs) of mapped small RNAs of length 21 and 24 nt in adjacent bins of 500 kb. For reads mapping at multiple locations, one random location was selected. Reads matching within predicted rRNA loci were ignored. Positions with more than 10 thousand mapped 21 nt sequences were labelled with the corresponding non-coding RNA prediction, if available, including the number of matching reads. Section 4 shows the chromosome-wide distribution of genomic variants in four sugar beet accessions and sea beet compared to RefBeet. Substitutions and deletions were detected by read-mapping with up to three variants per 100 nt read in 50 kb windows shifted by 25 kb. Shared and individual low-variation regions per accession are visible.



Extended Data Figure 2 | Genomic features of RefBeet chromosomes 6–9. For details see Extended Data Fig. 1.

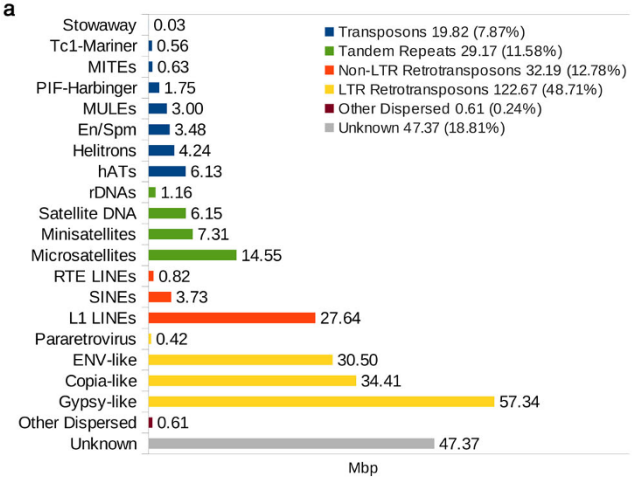


Extended Data Figure 3 | K-mer distribution and read coverage. **a**, Number of 17mers at different coverages. **b, c**, Correlation of read coverage and GC content of reads generated from a PCR-amplified library (**b**) and a PCR-free library (**c**). Read data sets in **b** and **c** were aligned against RefBeet. The GC content and the amount of aligned bases were computed in sliding windows of 500 bases shifted by 100 bases. To reduce the amount of data points only chromosome 1 scaffold 1 (Bvchr1.sca001, 8 Mb) was plotted.



Extended Data Figure 4 | Genetic vs physical distances. **a**, Genetic and physical positions of 983 genetic markers in the genetic map of sugar beet[2] and the RefBeet assembly, respectively. The expected physical distance in sugar beet had been reported as 855 kb per 1 cM, with deviations of up to 50-fold[2]. In RefBeet only 5% of marker pairs showed the expected physical distance (855 kb ± 20%) suggesting strict partitioning of the genome into regions favouring or disfavouring recombination events.

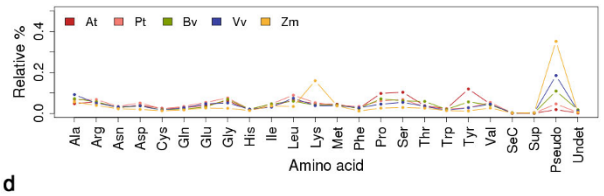
a



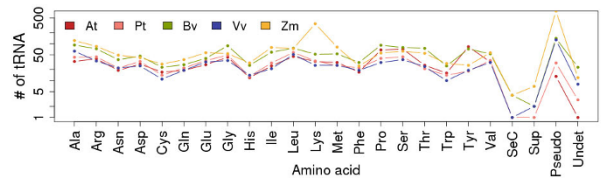
b

Class	Predicted	Covered by small RNA reads		families / subtypes
miRNA	522	258	49.43%	143
rRNA	232	231	99.57%	5
snRNA	148	121	81.76%	10
snoRNA	764	218	28.53%	115
tRNA	1,297	1,043	80.42%	103
other	42	22	52.38%	12
Total	3,005	1,893	1	388

c



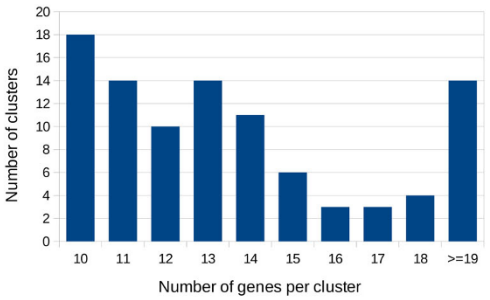
d



e

	Eudicots				Monocots
	Caryo-phyllales	Rosids			
Amino acid	***B. vulgaris***	*A. thaliana*	*P. tricho-carpa*	*V. vinifera*	*Z. mays*
Ala	92	33	43	63	122
Arg	73	39	44	34	85
Asn	37	19	22	22	49
Asp	46	28	33	25	42
Cys	23	17	14	11	28
Gln	27	19	22	19	37
Glu	40	27	35	32	57
Gly	88	43	49	35	54
His	26	12	13	14	30
Ile	59	25	30	21	79
Leu	76	45	58	51	74
Lys	52	33	34	26	349
Met	53	31	26	26	81
Phe	31	17	22	19	25
Pro	91	68	40	31	56
Ser	79	72	44	37	63
Thr	75	26	21	24	55
Trp	25	16	14	10	29
Tyr	72	83	18	19	26
Val	53	32	35	31	57
SeC	4	0	1	1	4
Sup	2	0	1	2	7
Pseudo	140	13	30	127	770
Undet	23	1	3	8	12
Total	1,124	685	619	553	1,409

f



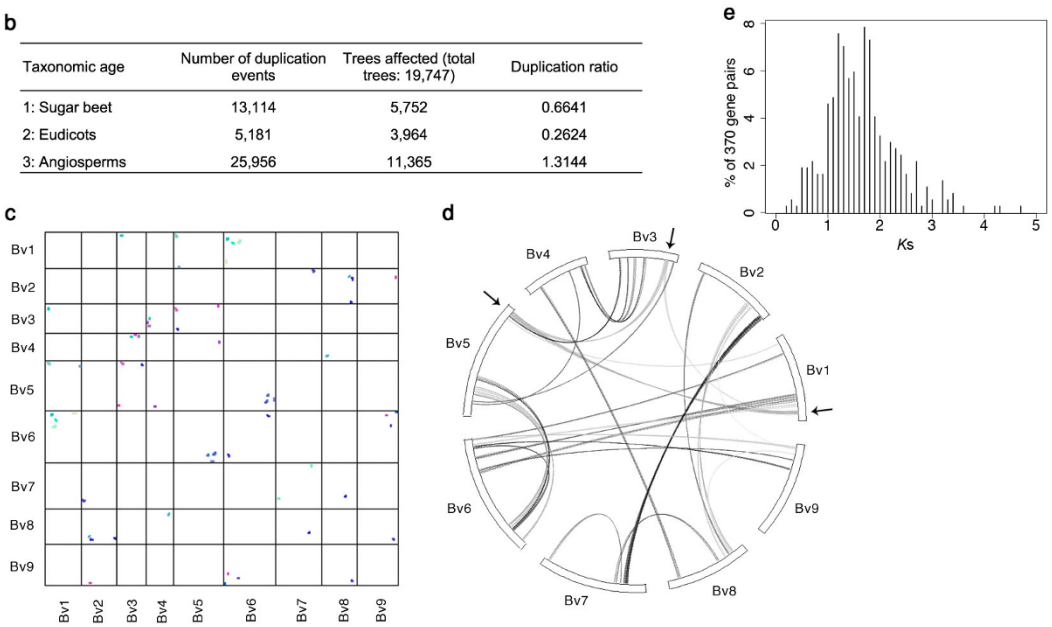
Extended Data Figure 5 | Annotation of repeats and non-coding RNA genes. **a**, Repeat content of the sugar beet genome assembly. A total of 252 Mb (42.3%) of the genome assembly consist of repetitive DNA with retrotransposons as the most abundant repeat fraction. All major superfamilies of DNA transposons were represented, showing a dispersed or slightly centromere-enriched distribution along the chromosomes. Microsatellites and minisatellites were well represented owing to flanking heterogeneous sequences, which allowed their assembly. The remaining repetitive sequences ('Unknown') in 459 families represent potentially new repeats, most likely rearranged or truncated retrotransposons. **b**, Summary of non-coding RNA gene annotations. For different classes (miRNA, microRNA; rRNA, ribosomal RNA; snRNA, spliceosomal RNA; snoRNA, small nucleolar RNA; tRNA, transfer RNA) the number of predictions, the number and percentage of predictions with overlapping small RNA reads, and the number of families/subtypes are listed. **c**, Proportion of annotated tRNAs by amino acid for the five species studied (At, *Arabidopsis thaliana*; Pt, *Populus trichocarpa*; Bv, *Beta vulgaris*; Vv, *Vitis vinifera*; Zm, *Zea mays*). **d**, Absolute numbers of annotated tRNAs by amino acid. Except for pseudogenes the proportion of tRNAs is relatively constant among all species (species names as in **c**). **e**, Number of annotated tRNAs by amino acid and species (as predicted by tRNAscan-SE). The total is computed without the last two rows containing pseudogenes and presumably defunct tRNAs with undetermined anti-codon. **f**, Number and size of *Beta vulgaris* gene clusters of at least 10 members representing expanded gene families. A total of 1,274 genes are contained in 97 clusters.

a

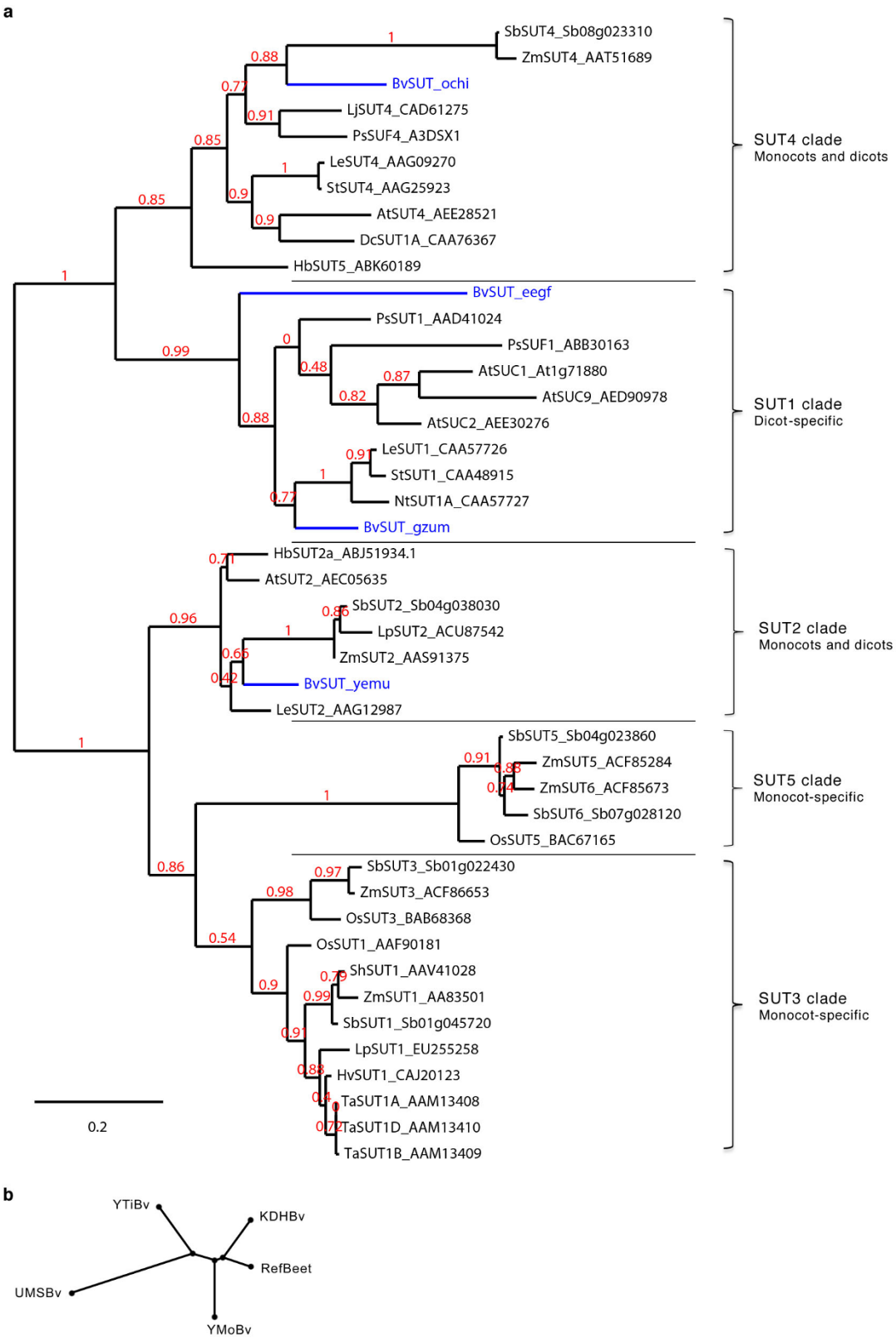
Phylogenetic trees analysed	*B. vulgaris*		Other plants			
	# orthologue	% orthologue	Species name	Total genes	# orthologue	% orthologue
12,109	16,681	60.96	*A. thaliana*	26,763	17,944	67.05
10,742	17,356	63.43	*Z. mays*	38,936	19,977	51.31
11,985	17,429	63.69	*S. tuberosum*	38,814	17,686	45.57
12,449	17,511	63.99	*V. vinifera*	26,278	15,102	57.47
11,106	18,038	65.92	*O. sativa*	40,548	18,478	45.57
13,449	18,909	69.10	*G. max*	46,103	33,245	72.11
13,284	19,094	69.78	*S. lycopersicum*	34,635	19,032	54.95
13,833	19,732	72.11	*P. trichocarpa*	40,463	24,455	60.44
14,113	20,459	74.77	*T. cacao*	46,125	17,187	37.26

b

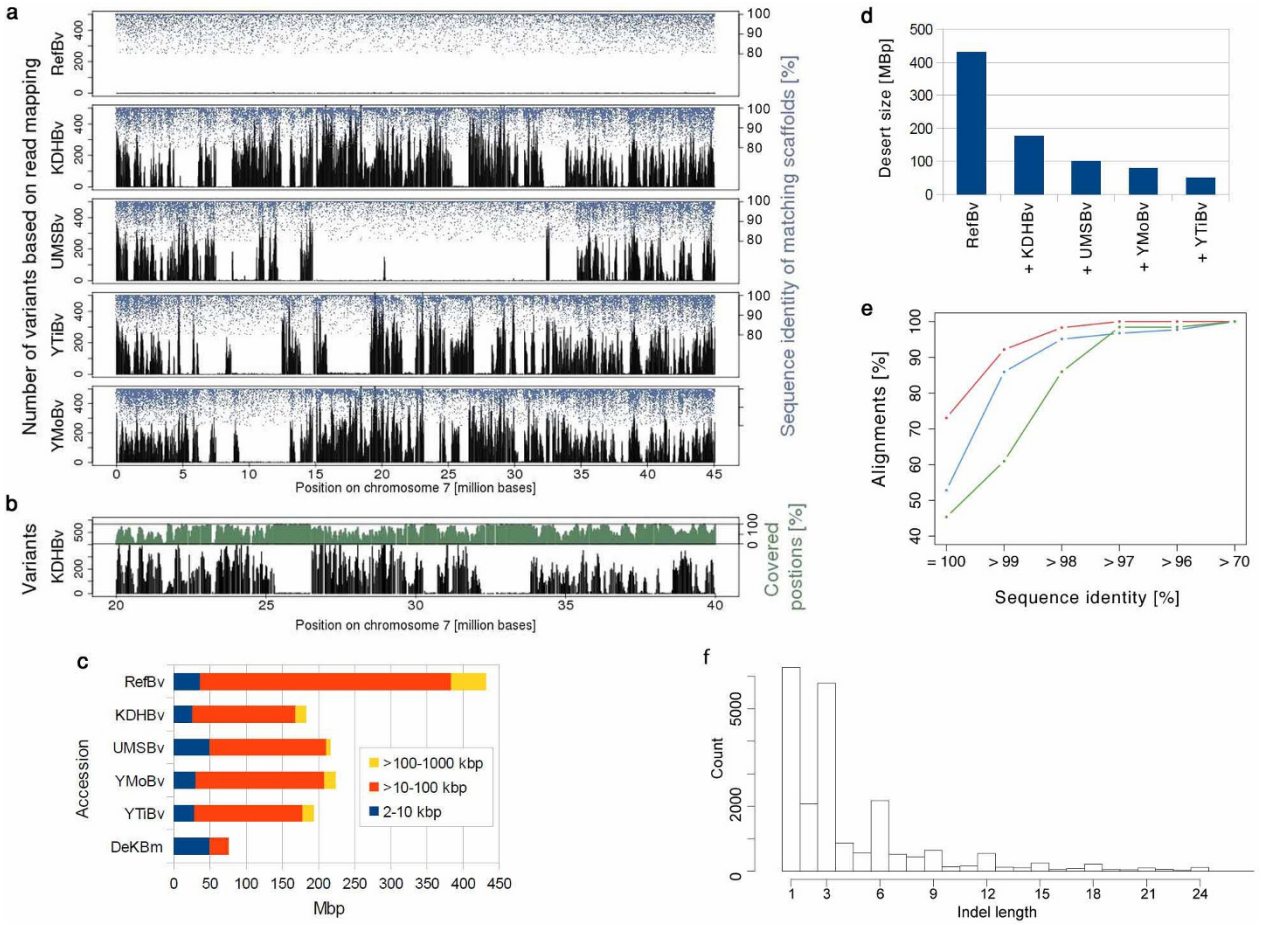
Taxonomic age	Number of duplication events	Trees affected (total trees: 19,747)	Duplication ratio
1: Sugar beet	13,114	5,752	0.6641
2: Eudicots	5,181	3,964	0.2624
3: Angiosperms	25,956	11,365	1.3144



Extended Data Figure 6 | Analysis of paralogous and orthologous genes. **a**, Number and percentage of detected orthologues between *Beta vulgaris* and nine other plant species. Orthology relationships with 10 or more proteins for any of the species were discarded in order to avoid biases introduced by species-specific gene family expansions. In total, 18,927 sugar beet genes had orthologues in at least one of nine plants, and 16,062 paralogous sugar beet genes appeared in 14,852 trees. **b**, Number of duplication events detected in gene trees grouped into three age classes. The duplication ratio was calculated as the number of age class-specific duplication events divided by the total number of trees containing duplication events. **c**, **d**, Collinear blocks of protein coding genes in *Beta vulgaris* as dotplot (**c**) or circular plot (**d**). Each dot or connecting line represents one gene pair, respectively. Shown are 34 collinear blocks containing 7–35 gene pairs. A triplicated region is visible on chromosomes 1, 3 and 5 (arrows). **e**. Histogram of *Ks* values for *Beta vulgaris* protein coding gene pairs in collinear blocks. *Ks* values mainly scatter between 1.2 and 1.8 and show peaks at 1.2 and 1.7.



Extended Data Figure 7 | Phylogenetic trees. **a**, Phylogenetic tree of 44 sucrose transporter protein sequences in higher plants including *Beta vulgaris*. The reliability for internal branches is indicated in red ranging from 0 = unreliable to 1 = highly reliable (aLRT statistics). At, *Arabidopsis thaliana*; Bv, *Beta vulgaris*; Dc, *Daucus carota*; Hb, *Hevea brasiliensis*; Hv, *Hordeum vulgare*; Le, *Lycopersicum esculentum* renamed *Solanum lycopersicum*; Lj, *Lotus japonicus*; Lp, *Lolium perenne*; Nt, *Nicotiana tabacum*; Os, *Oryza sativa*; Ps, *Pisum sativum*; Sh, *Saccharum* Hybrid Cultivar Q117; St, *Solanum tuberosum*; Sb, *Sorghum bicolor*; Ta, *Triticum aestivum*; Zm, *Zea mays*. **b**, Intraspecific relationship of five *Beta vulgaris* accessions based on the alignment of 2,112 shared genes.



Extended Data Figure 8 | Intraspecific variation. **a**, Number of variants inferred from read mapping (black) and sequence identity of matching scaffolds (blue) along RefBeet chromosome 7. The variation profiles of five different accessions including the reference accession (RefBv) are shown. Regions with a high number of read-mapping variants showed a higher density of scaffolds of low sequence identity. However, low-identity scaffolds were also present in low-variation regions of mapped reads. **b**, Detailed view of the distribution of read mapping variants and read coverage (green) in *Beta vulgaris* accession KDHBv compared to RefBeet. The secondary *y* axis on the right side indicates the percentage of positions per window covered in the alignment. Low-variation regions were generally well covered. **c**, Fraction of variation deserts of different lengths along RefBeet based on read-mapping of genomic data sets and alignment of assembled scaffolds. The six different genotypes include the reference, four other *Beta vulgaris* accessions, and one *Beta maritima* accession. Variation deserts were found in all chromosomes. The variation deserts of non-reference sugar beet accessions contained 49% (179 Mb in KDH) to 58% (217 Mb in YMo) of all covered RefBeet positions. **d**, Intersection of variant deserts. Starting from RefBv, the size of shared variant deserts decreased by including additional *Beta vulgaris* accessions. **e**, Sequence conservation comparison of three groups of genes. Genes with GO term enrichment localized within variation deserts shown in red; genes without GO term enrichment localized within variation deserts shown in blue, genes localized outside of variation deserts shown in green. For each of the three groups 17 randomly selected genes with confirmed exon–intron structure were aligned to 24 additional sugar beet accessions. The sequence conservation was determined from the identity of the sequence alignment. Genes with GO term enrichment localized within variation deserts had the highest fraction of high identity gene alignments, followed by genes without GO term enrichment localized within variation deserts. **f**, Length distribution of insertions and deletions in coding sequences. Apart from one-base indels, indels of length three or multiples of three (3*n*) were overrepresented. Of all genes affected by indels, 49.1% had a single 3*n* indel and 5.0% had more than one indel (any length) with bases summing up to 3*n*.

Extended Data Table 1 | Sequencing data, assembly results and plant species

a

Assembly	Single reads [million]	Read pairs [million]	Sequence coverage	Library coverage
RefBeet	43.7	66.3	30.2	368.4
RefBv	85.8	593.0	155.5	578.6
KDHBv	27.6	259.1	60.8	356.9
UMSBv	62.0	379.0	99.9	473.1
YMoBv	36.1	267.9	67.1	320.2
YTiBv	34.4	271.3	67.9	368.9
DeKBm	102.2	716.1	184.2	945.3
Spinach	31.2	344.5	65.1	376.0

b

Accession	RNA type	Single reads [million]	Read pairs [million]
KWS2320	mRNA	33.64	181.62
KWS2320	small RNA	28.45	324.68
KDHBv	mRNA	-	12.23
UMSBv	mRNA	-	14.35
U1Bv	mRNA	-	32.4
YMoBv	mRNA	-	22.83
YTiBv	mRNA	-	27.9

c

Assembly	Size [Mbp]	Number of sequences	N50 size [kbp]	Predicted genes
RefBeet	569.0	43,721	1,685	27,421
RefBv	508.2	35,775	76	25,813
KDHBv	490.8	38,192	70	25,368
UMSBv	552.4	88,870	46	31,355
YMoBv	469.6	50,310	39	25,927
YTiBv	486.5	49,232	55	25,626
Spinach	499.9	104,270	19	-

d

Taxonomic group	Taxonomic subgroup	Species name	Species short name	Number of genes	Unique sequences	Source	Version	As in
Eudicots	Caryophyllales	*Beta vulgaris* ssp. *vulgaris*	*Beta vulgaris* or sugar beet	27,421	27,364	this work	RefBeet-1.1	11/01/12
		Beta vulgaris ssp. *maritima*	*Beta maritima* or sea beet					
		Spinacia oleracea	spinach					
	Rosids	*Arabidopsis thaliana*	Arabidopsis	28,128	26,763	Quest For Orthologs RELEASE 2011/04	2011_04	07/01/11
		Glycine max	soybean	46,369	46,103	Phytozome v7.0	109	07/01/11
		Populus trichocarpa	poplar	40,670	40,463	Phytozome v7.0	156	07/01/11
		Theobroma cacao	cacao	46,229	46,125	CocoaGen DB	1	07/01/11
		Vitis vinifera	grapevine	26,346	26,278	Phytozome v7.0	145	07/01/11
	Asterids	*Solanum lycopersicum*	tomato	34,725	34,635	International Tomato Annotation Group	ITAG 2.3	02/01/12
		Solanum tuberosum	potato	39,032	38,814	Potato Genome Sequencing Consortium	PGSC 3.4	02/01/12
Monocots		*Oryza sativa* ssp. *indica*	rice	40,745	40,548	ENSEMBL - Plants	Jan_2005	07/01/11
		Zea mays	maize	39,656	38,936	maizesequence.org	ZmB73_5b	07/01/11

a, Assembly input of genomic DNA data after quality-filtering. Number of input reads and their genome coverage. DeKBm: *Beta maritima*; Spinach: *Spinacia oleracea*; others: *Beta vulgaris*. RefBeet and RefBv data were generated from the reference genotype KWS2320. **b**, RNA data after quality-filtering. RNA data used as evidence for coding and non-coding gene predictions in *Beta vulgaris* assemblies. **c,** Assembly results. Summary of assembly statistics and number of predicted genes. The number of sequences is the number of scaffolds plus the number of contigs that remained unscaffolded of size 500 bp or larger. The N50 size refers to this set of sequences. **d,** Species names and data sources of plant species used in comparative studies.