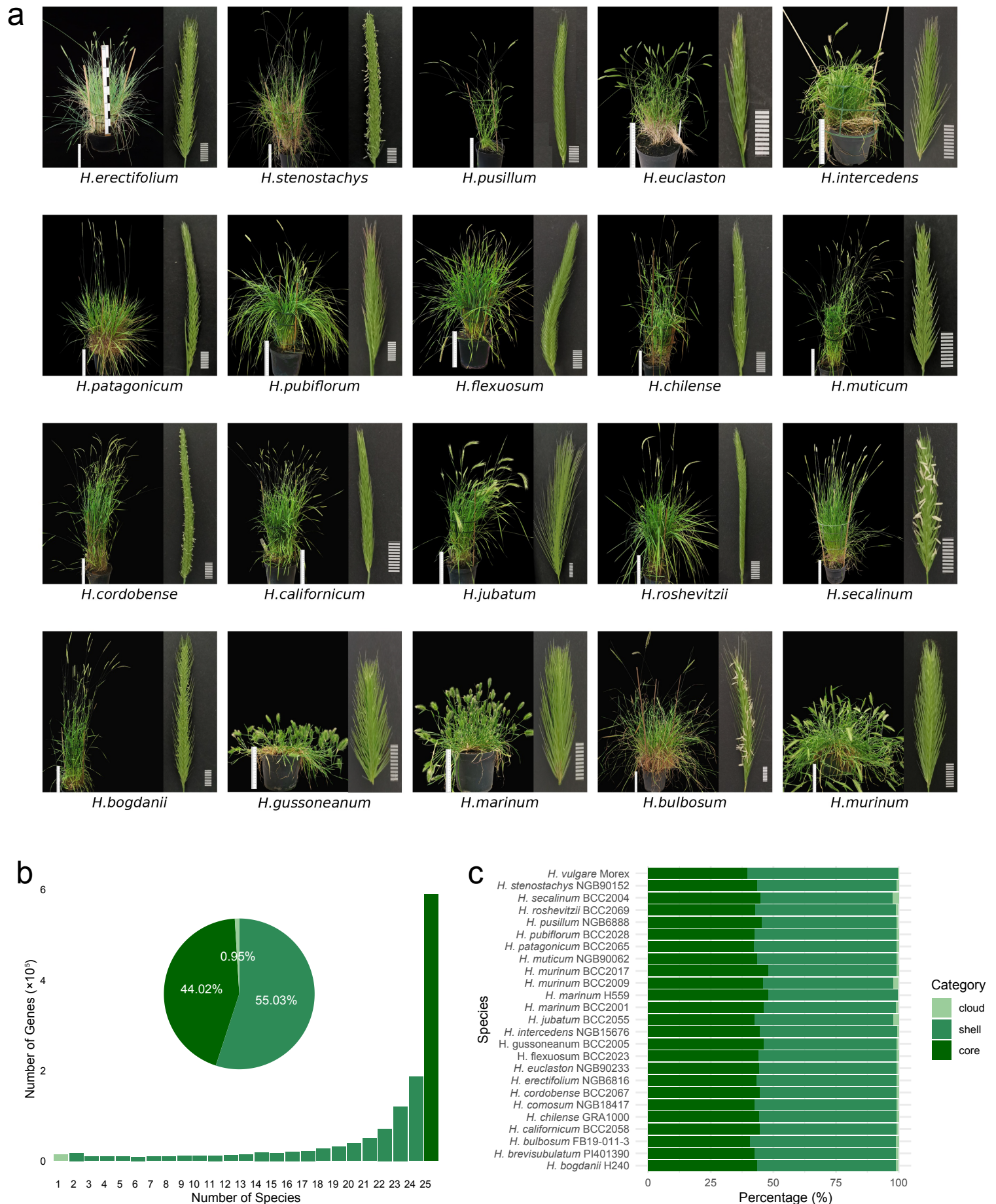
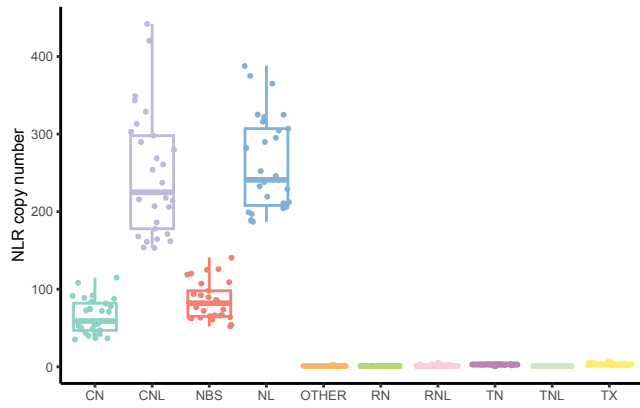


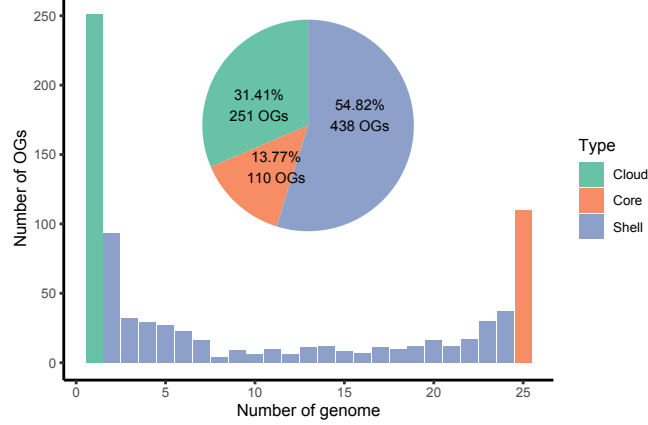


**Extended Data Figure 1: Morphological and genomics diversity across *Hordeum* species.** (a) Representative images showing whole plant architecture and spike morphology for 20 species in the genus *Hordeum*. Species shown (left to right, top to bottom): *H. erectifolium*, *H. stenostachys*, *H. pusillum*, *H. euclaston*, *H. intercedens*, *H. patagonicum*, *H. pubiflorum*, *H. flexuosum*, *H. chilense*, *H. muticum*, *H. cordobense*, *H. californicum*, *H. jubatum*, *H. roshevitzii*, *H. secalinum*, *H. bogdani*, *H. gussoneanum*, *H. marinum*, *H. bulbosum*, and *H. murinum* subsp. *glaucum*. Each panel displays a whole plant (left) and a close-up of the spike (right), highlighting variation in growth habit and inflorescence morphology across the genus. Note that the scale bars differ: the scale bar for whole plant images represents 16 cm, while the scale bar for spike images represents 1 cm. (b) Pie-chart providing ratios of conserved- and variable- genes for all *Hordeum* pangenome species. Bar chart illustrating the number of genes shared between different combinations of species within the *Hordeum* pangenome. Genes shared by all 25 species constitute the core genome while genes private to a single species are referred to as the cloud genome. (c) bar chart illustrating the proportion of genes contained in the core-, shell-, and cloud- gene portions by individual species.

a



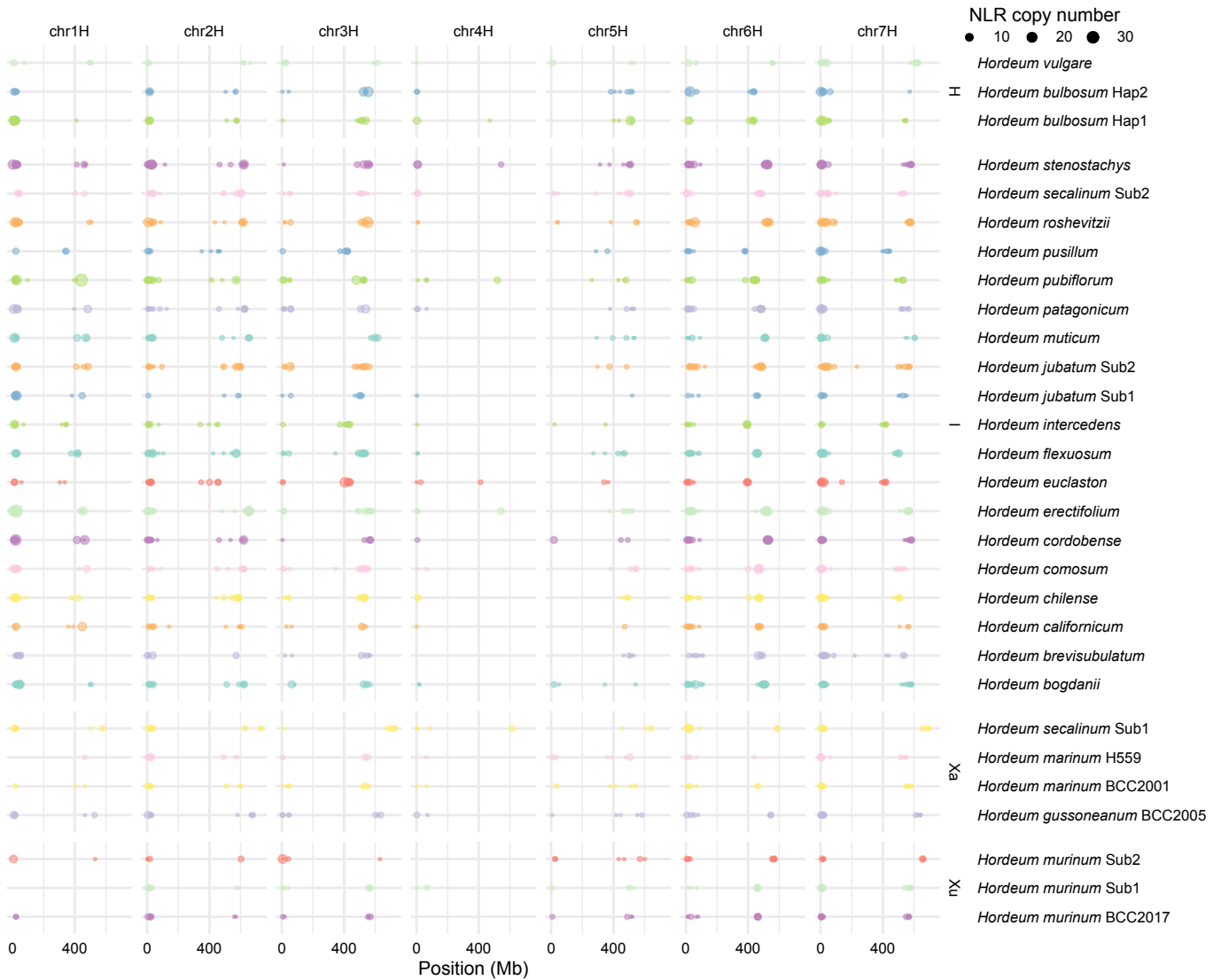
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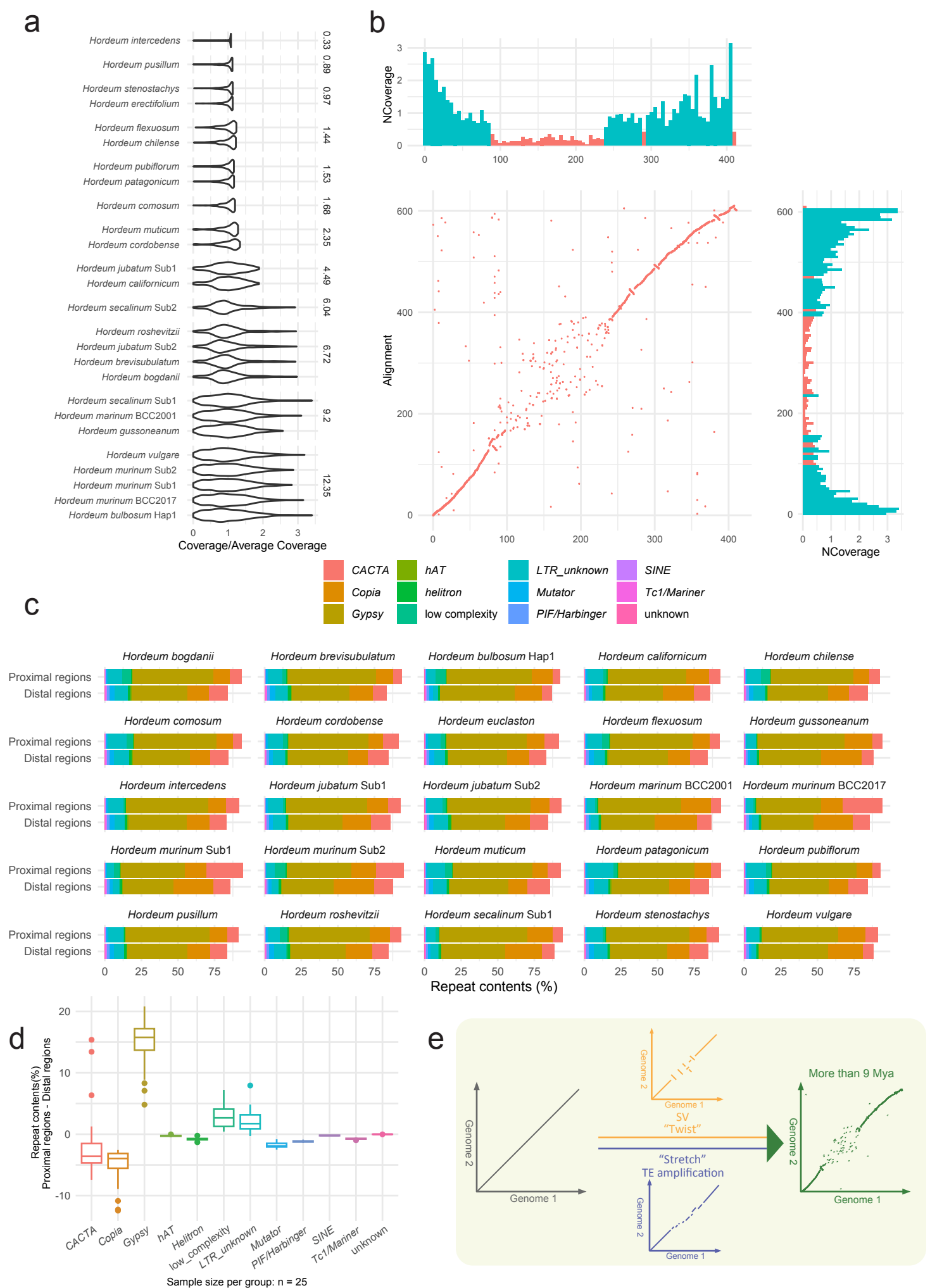
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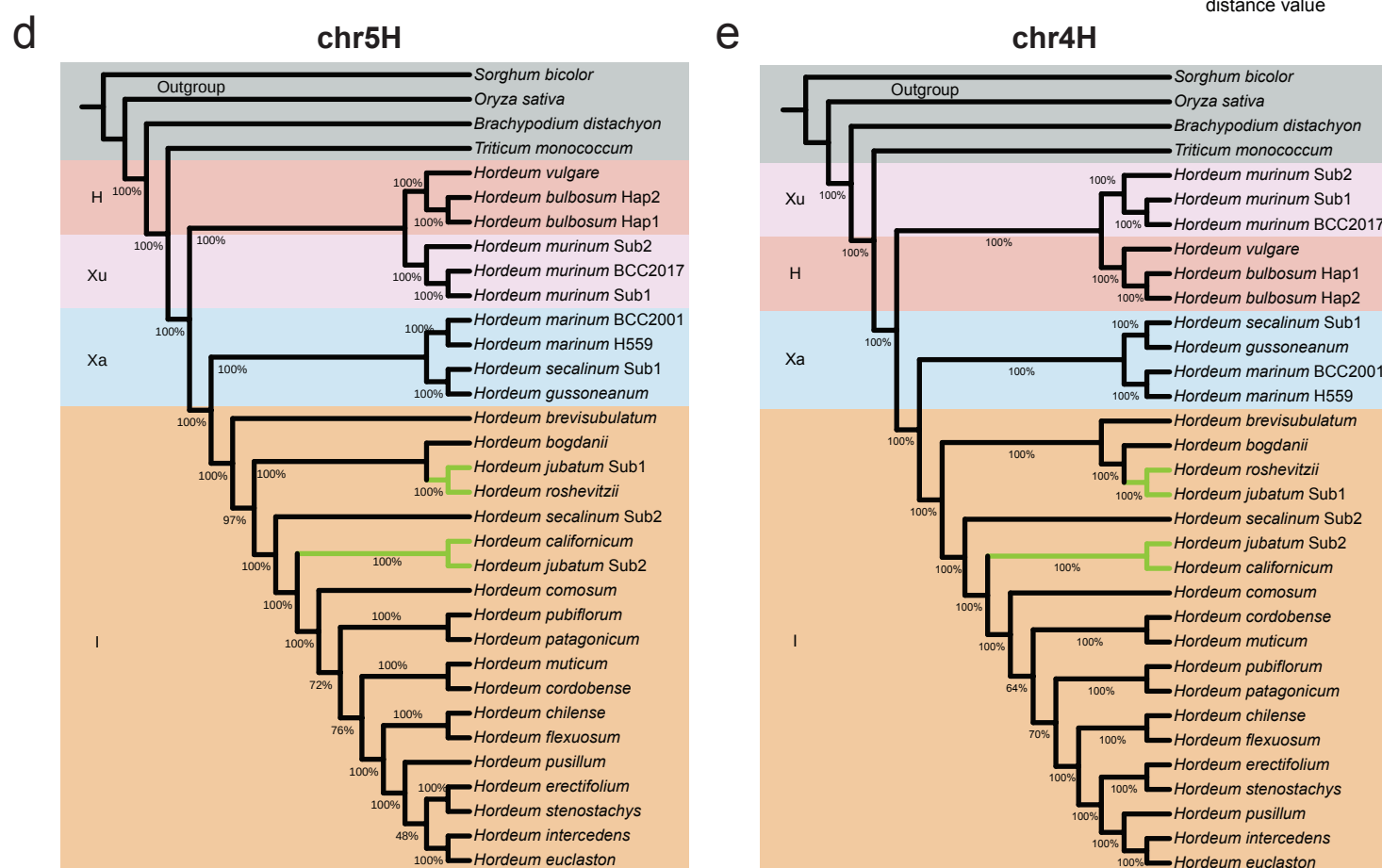
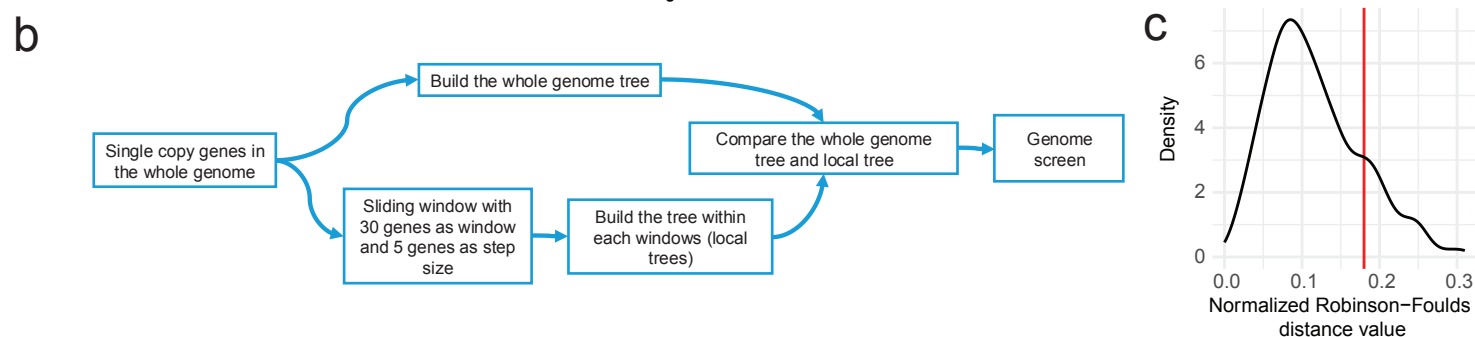
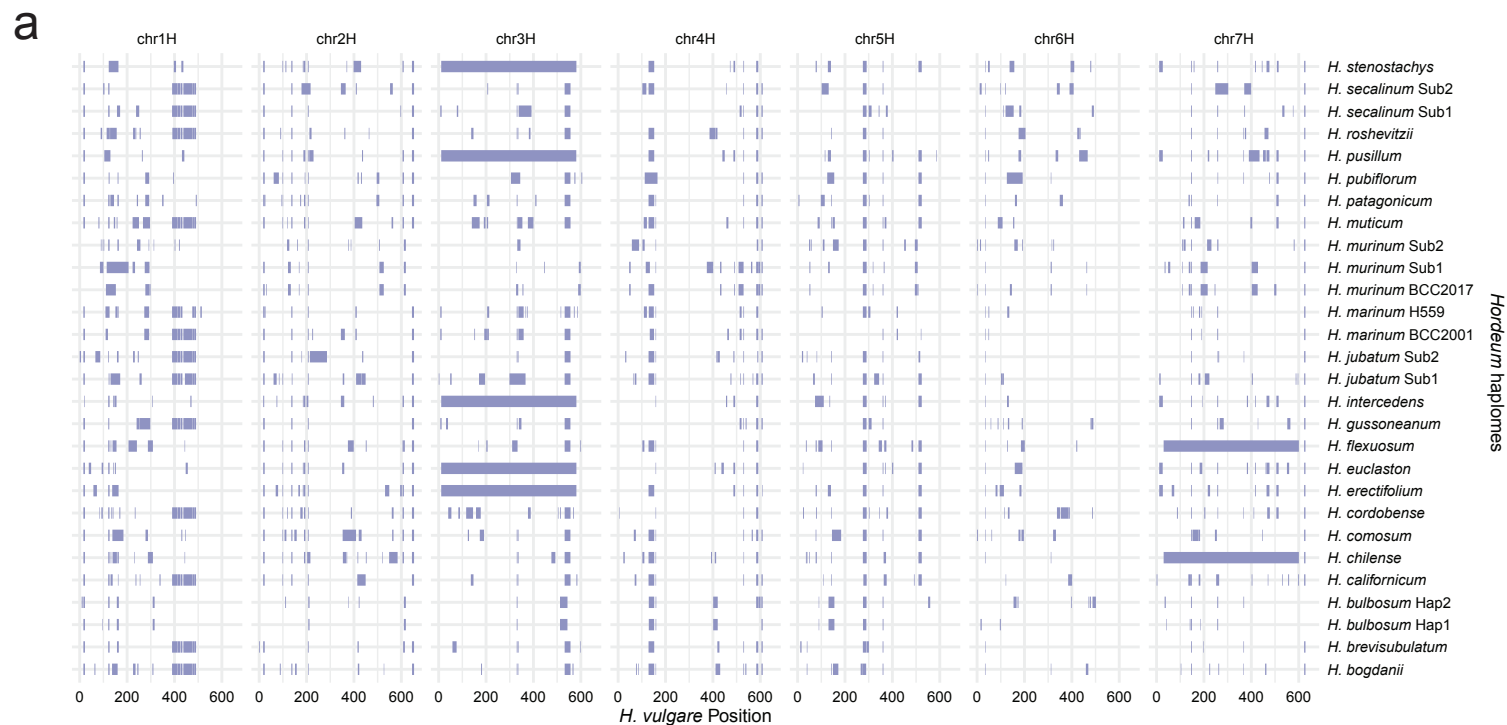


**Extended Data Figure 2: Landscape of NLR genes in the genus *Hordeum*.** **(a)** NLR gene copy numbers across 29 *Hordeum* haplomes. The central line in each boxplot represents the median; the box spans the interquartile range (IQR), and whiskers extend to  $1.5 \times \text{IQR}$ . TNL: Contain TIR, NBS, and LRR domains. CNL: Contain CC, NBS, and LRR domains. RNL: Contain RPW8, NBS, and LRR domains. RN: Contain RPW8 and NBS domains only. NL: Contain NBS and LRR domains, but lack TIR, CC, and RPW8. TN: Contain TIR and NBS domains only. CN: Contain CC and NBS domains only. NBS-only: Contain only the NBS domain. TIR = Toll/Interleukin-1 Receptor-like; NBS = Nucleotide-Binding Site; LRR = Leucine-Rich Repeat; CC = Coiled-Coil; RPW8 = Resistance to Powdery Mildew 8-like domain. **(b)** Comparison of NLR copy numbers among *Hordeum* genomes. Welch's t-test revealed a highly significant difference between the I genome group and other genome groups ( $p \text{ value} = 5.06 \times 10^{-7}$ ). **(c)** Bar chart showing the number of accessions represented in each NLR orthogroup (OG). The x-axis indicates accession count per OG. The pie chart summarizes conserved vs. variable genes across 25 accessions. **(d)** Distribution of NLR gene clusters ( $\geq 3$  genes) across 29 haplomes. Dot size represents the number of genes per cluster. The chr4H/chr5H group showed significantly different values compared to other chromosomes (Wilcoxon test,  $p \text{ value} < 2.2\text{e-}16$ ).



**Extended Data Fig. 3: “Stretch and twist” model of proximal region evolution. (a)** Normalized coverage density plot in *H. euclaston*. The right shows species ordered by divergence time; two distinct peaks appear among species that diverged over 9 million years ago. **(b)** Comparison of chromosome 4H between *H. vulgare* and *H. euclaston*. The bar plots along the x- and y-axes represent normalized coverage in 5 Mb windows. Coverage was calculated based on whole-genome alignment between *H. vulgare* and *H. euclaston*. Proximal and distal regions were defined using a normalized coverage threshold of 0.5. **(c)** Proportion of repeat types in proximal and distal regions. **(d)** Boxplot showing the difference in repeat composition between distal and proximal regions. **(e)** A schematic diagram of the “stretch-and-twist” model.



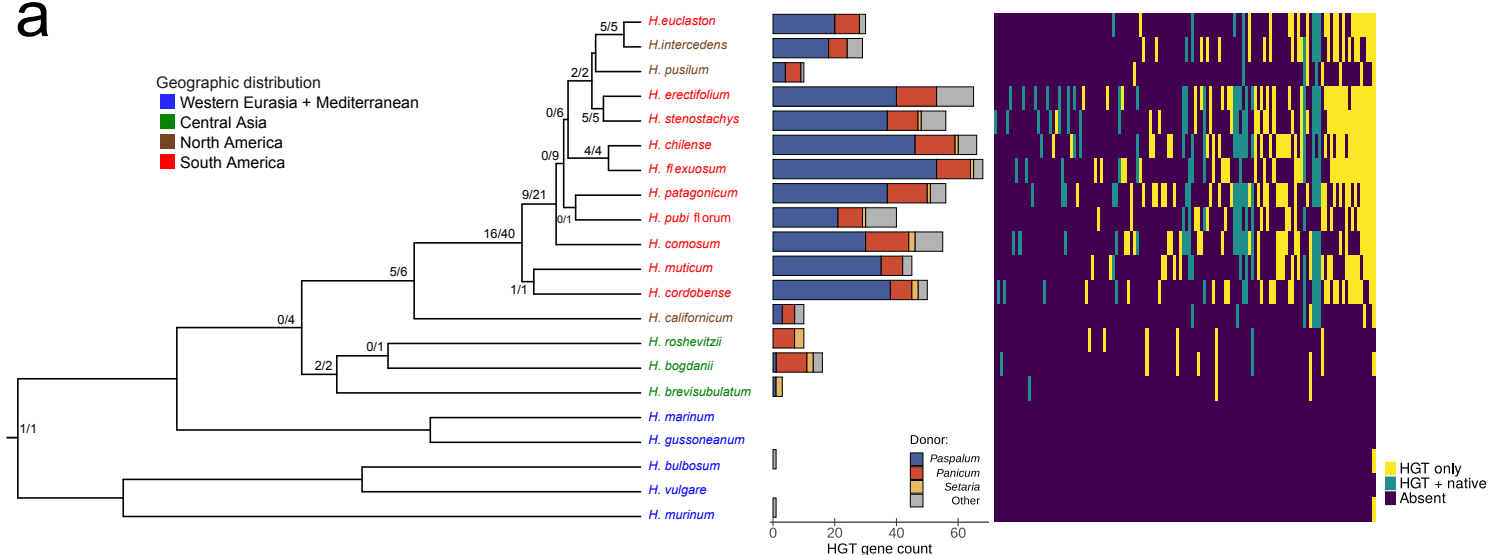


**Extended Data Fig. 4: Detection of deep hybridization events and segmental exchanges.** (a) Genome-wide distribution of distal inversions ( $\geq 2$  Mb) across *Hordeum* haplotypes. (b) Schematic overview of the hybridization scanning method. (c) Density distribution of normalized Robinson–Foulds (RF) distances; red line (0.18) separates two peaks. (d) Phylogenetic tree constructed from single-copy genes located in a segmental exchange region on chromosome 5H; bootstrap values shown at each node. The green branches show where the changes occurred compared to the reference tree. Approximately Unbiased (AU) test based on concatenated local single-copy genes supports the local tree ( $p\text{-AU} = 1.0$ ) and rejects the reference tree ( $p\text{-AU} = 6.11 \times 10^{-12}$ ). (e) Analysis of the segmental exchange region on chromosome 4H, performed using the same approach. Approximately Unbiased (AU) test based on concatenated local single-copy genes supports the local tree ( $p\text{-AU} = 1.0$ ) and rejects the reference tree ( $p\text{-AU} = 1.11 \times 10^{-37}$ ).

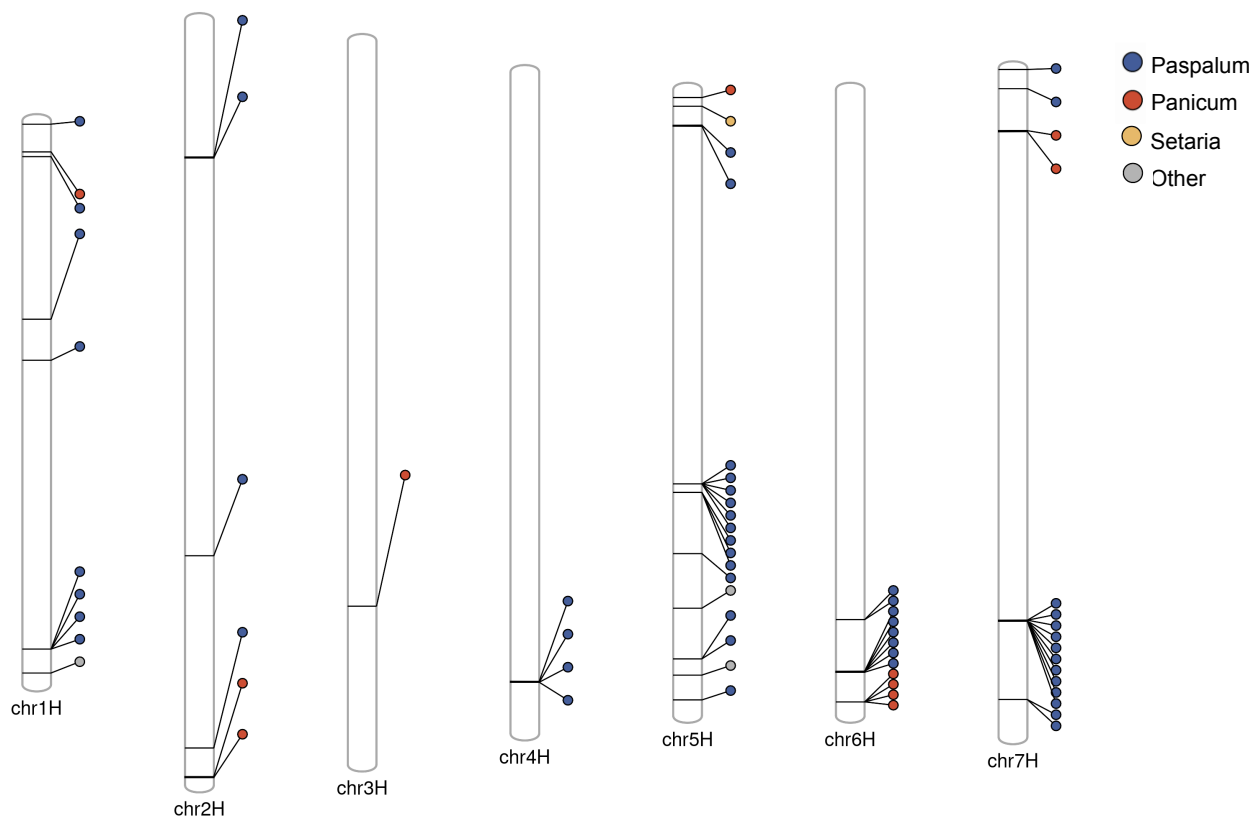
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Geographic distribution

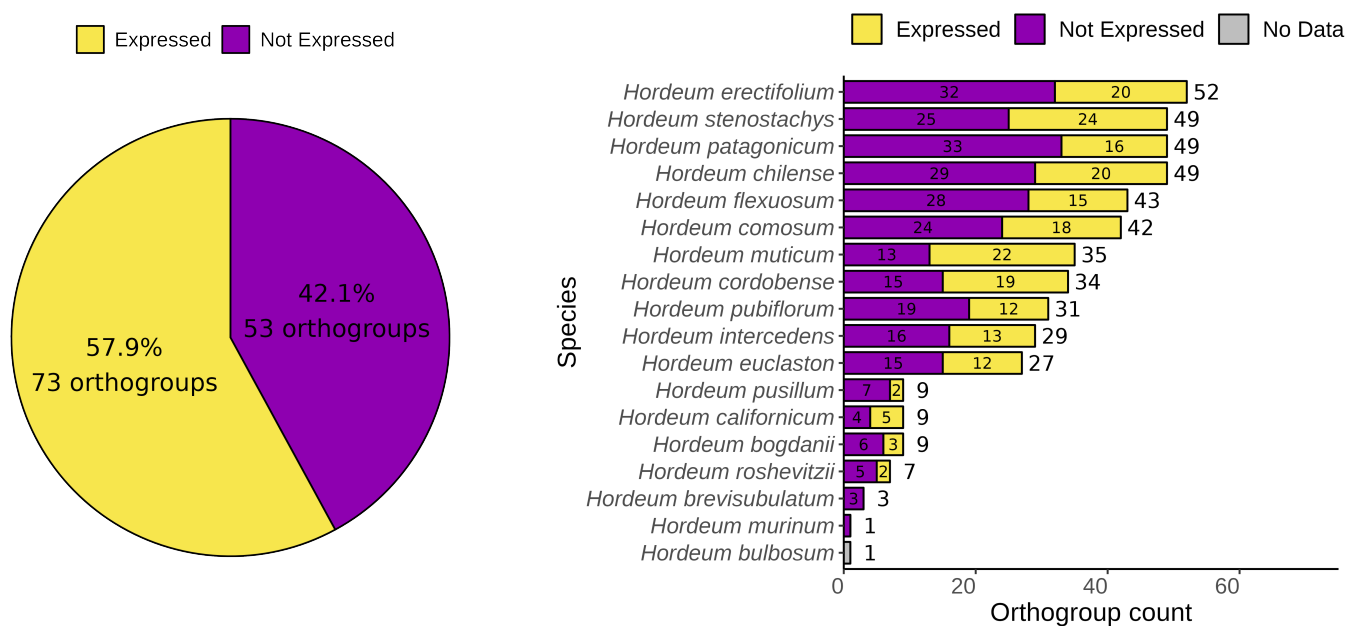
- Western Eurasia + Mediterranean
- Central Asia
- North America
- South America



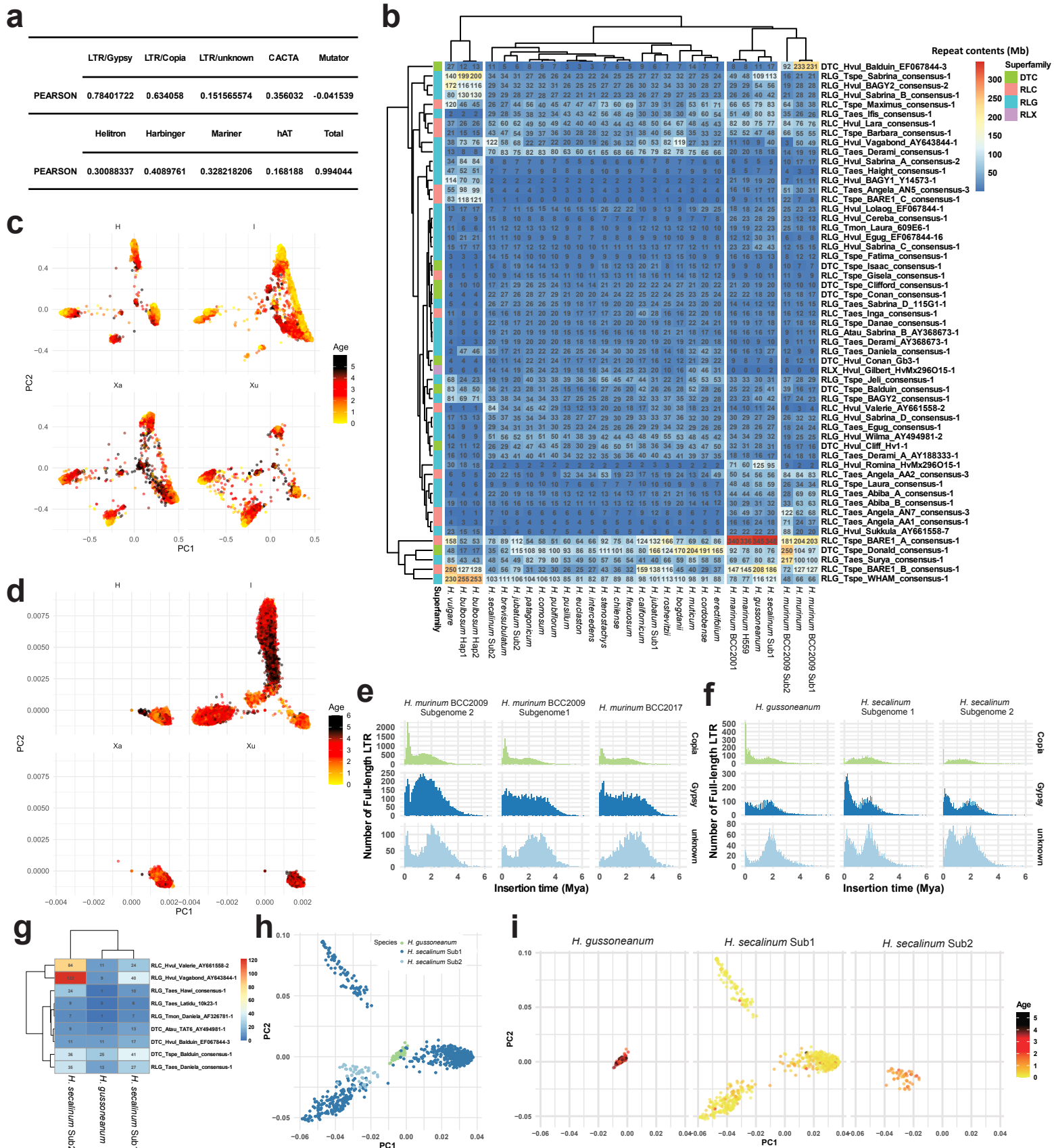
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c

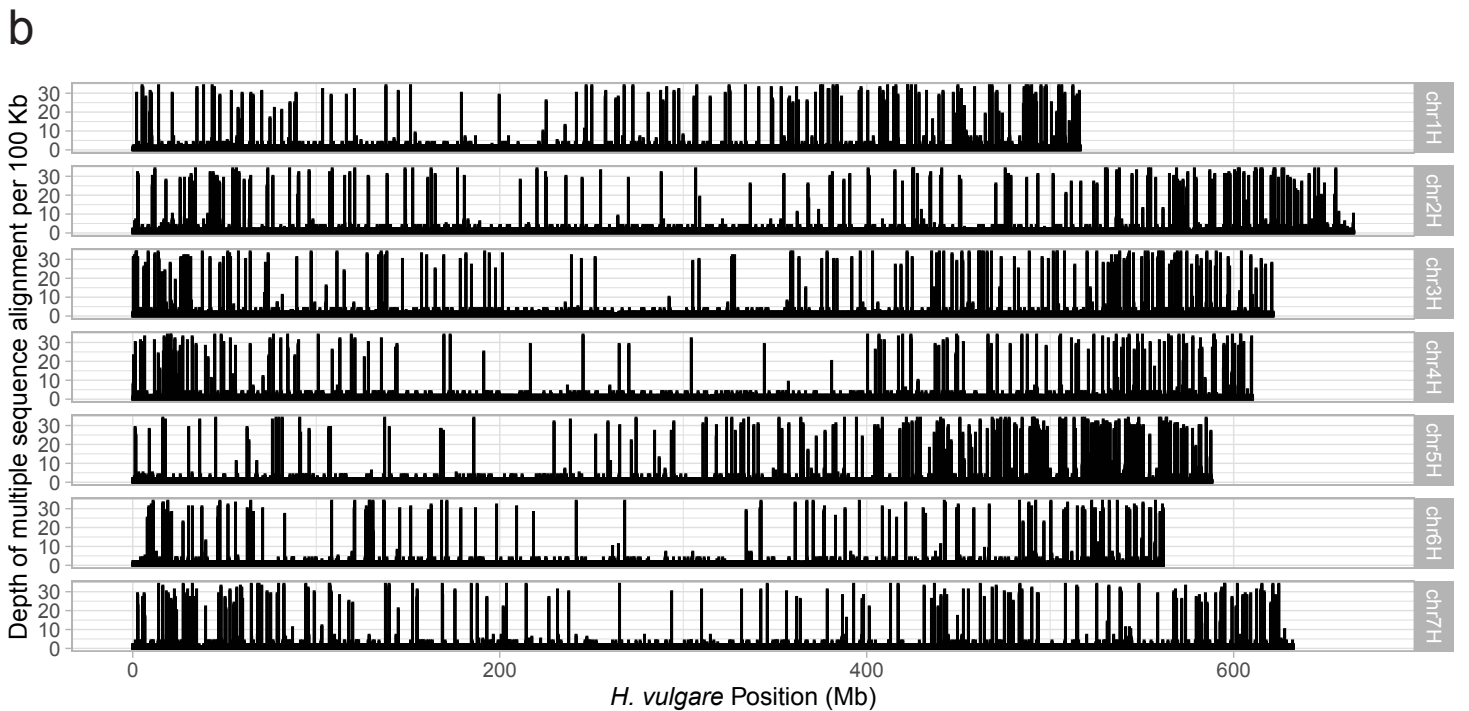
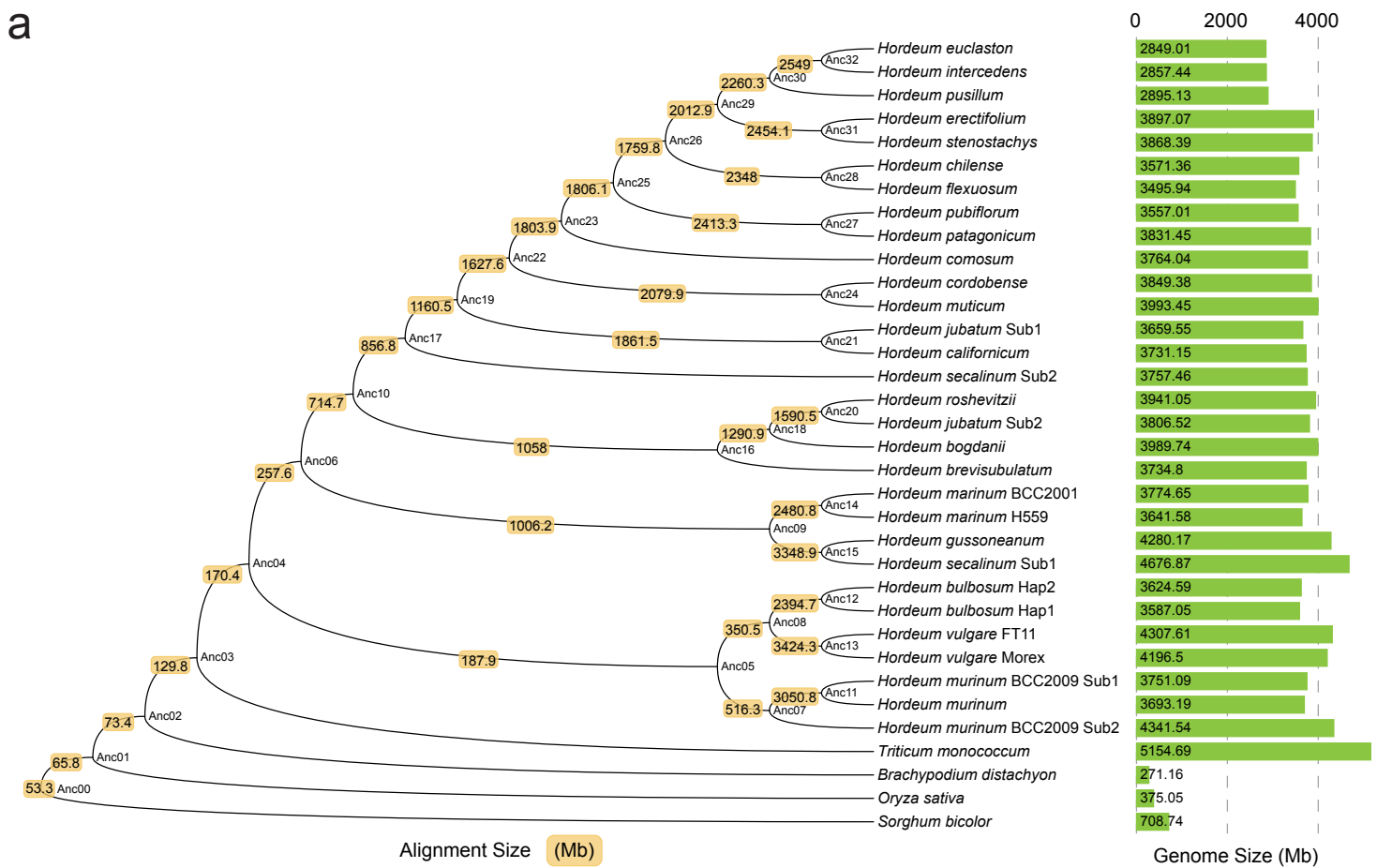


**Extended Data Fig. 5: Horizontal gene transfer (HGT) from Panicoideae into *Hordeum* species.** (a) Overview of HGT presence across diploid species of *Hordeum* genus. Left panel: Phylogenetic tree of the genus. Numbers adjacent to the nodes indicate the number of HGT in the respective ancestors: the first value reflects ancestral state reconstruction analysis, the second value represents manual correction (see Online methods). Geographical distribution adapted from Brassac & Blattner<sup>2</sup> Central panel: Number of panicoid genes acquired by HGT in *Hordeum* species, categorized by donor genus: *Paspalum* (blue), *Panicum* (red), *Setaria* (yellow), and other Panicoideae grasses (grey). Right panel: Distribution of panicoid HGT orthogroups across *Hordeum* species: absent (purple), present (yellow), and present with a native copy in the *Hordeum* pan-genome (green). (b) Chromosomal distribution of foreign genes in *H. flexuosum*. Dots represent individual HGT genes from donor genus: *Paspalum* (blue), *Panicum* (red), *Setaria* (yellow), and other Panicoideae grasses (grey). (c) Expression of HGT. Left panel: Percentage of orthogroups with at least one expressed HGT gene (TMM  $\geq 1$ ) in at least one tissue (yellow); no expression in all tissues and species (purple). Right panel: Expression in individual species. The number of orthogroups with at least one expressed HGT gene (TMM  $\geq 1$ ) in at least one tissue (yellow); no expression in all tissues (purple); species with no RNA-seq data available (grey).



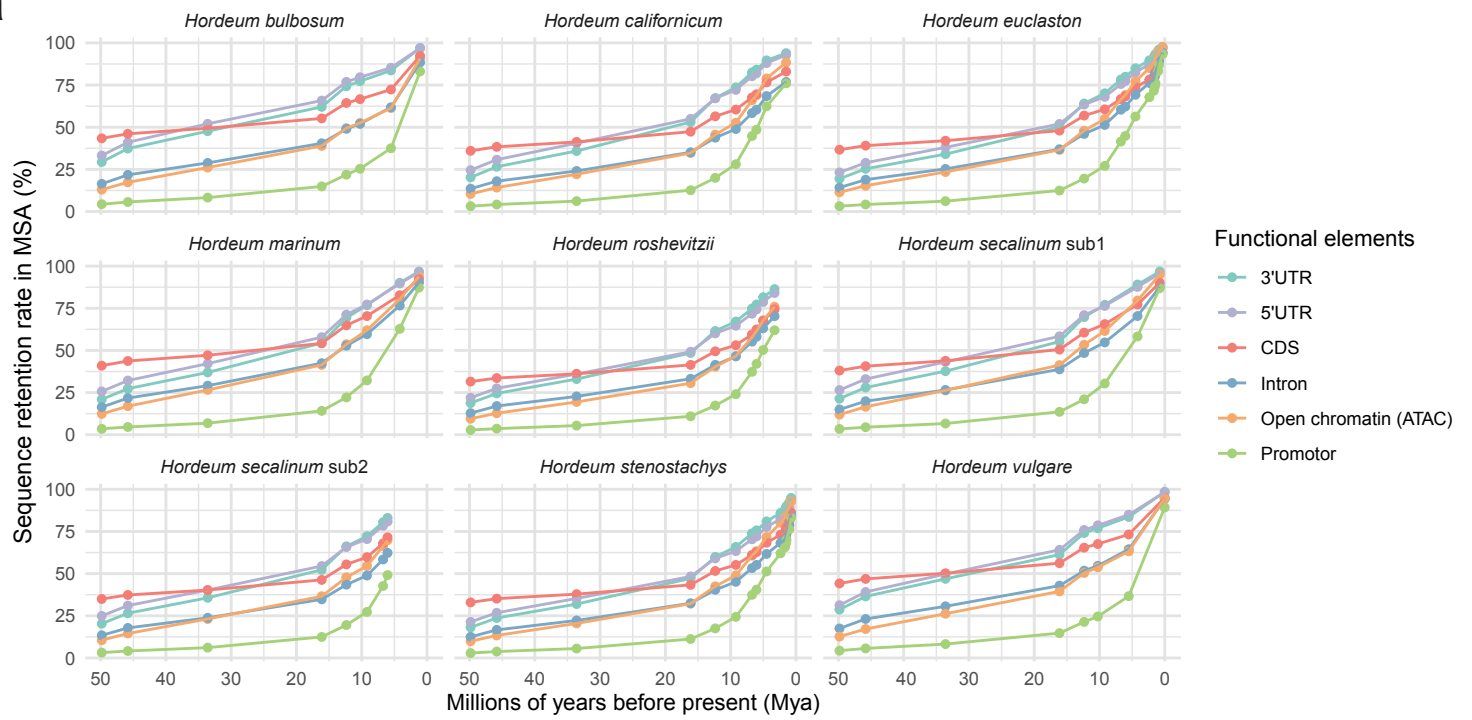
**Extended Data Fig. 6: Transposon dynamics underlie genome expansion in *Hordeum*.** (a) Pearson correlation coefficients between TE superfamilies and genome size. (b) Heatmap showing abundance of 55 TREP TEs (>10 Mb average cumulative length). The numbers in each cell indicate the cumulative size in Mb of all elements assigned to a family. (c) PCA of 334,142 full-length *RLC\_BARE1* elements; each dot represents one element, colored by insertion time. (d) PCA of 254,001 full-length *RLG\_Sabrina* elements. (e) Insertion time distributions of full-length LTR elements in *H. secalinum* and *H. gussoneanum*; the latter is the diploid progenitor of the former. (f) Insertion time distributions of LTR elements in *H. murinum* BCC2009 (subgenome 1) and its diploid progenitor BCC2017. (g) Heatmap showing TE families expanded in *H. secalinum* subgenome 1 relative to *H. gussoneanum*. (h) PCA of 1,041 full-length *RLG\_Sabrina* elements; colors represent genomes. (i) PCA colored by estimated insertion time.



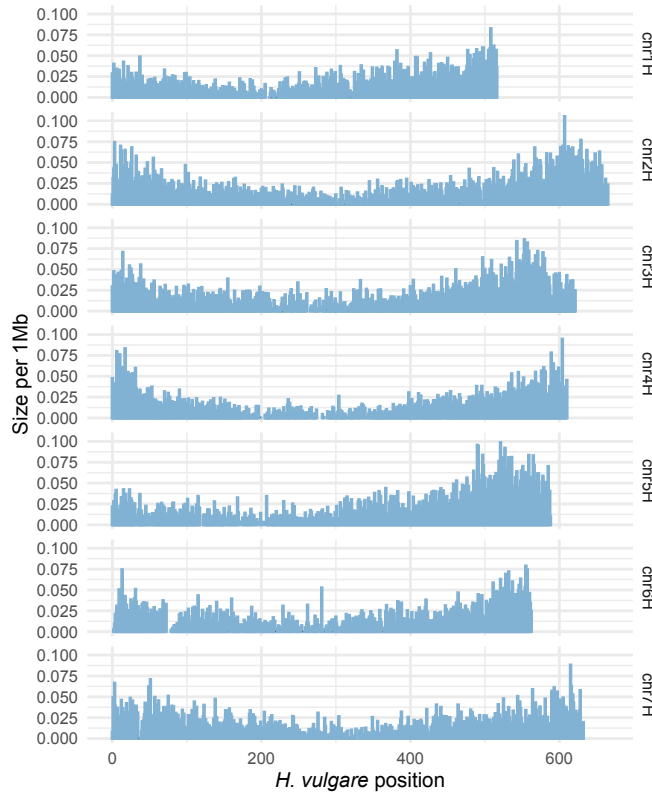


**Extended Data Fig. 7: Whole-genome alignment of 30 *Hordeum* and 4 outgroup haplomes. (a)** Summary of genome-wide alignments among 30 *Hordeum* and 4 outgroup haplomes. Left: species tree with cumulative alignment length at each node; right: bar plots showing aligned genome size for each species. **(b)** Distribution of alignment depth per 100-kb window based on *H. vulgare*.

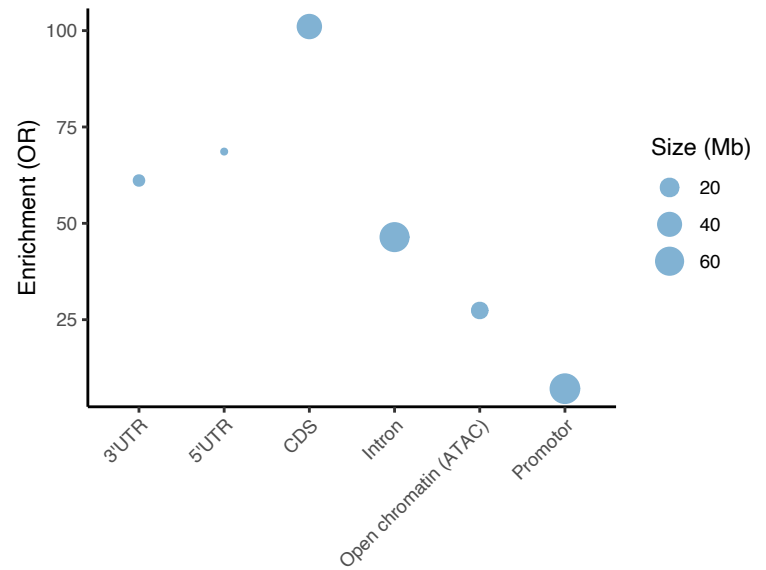
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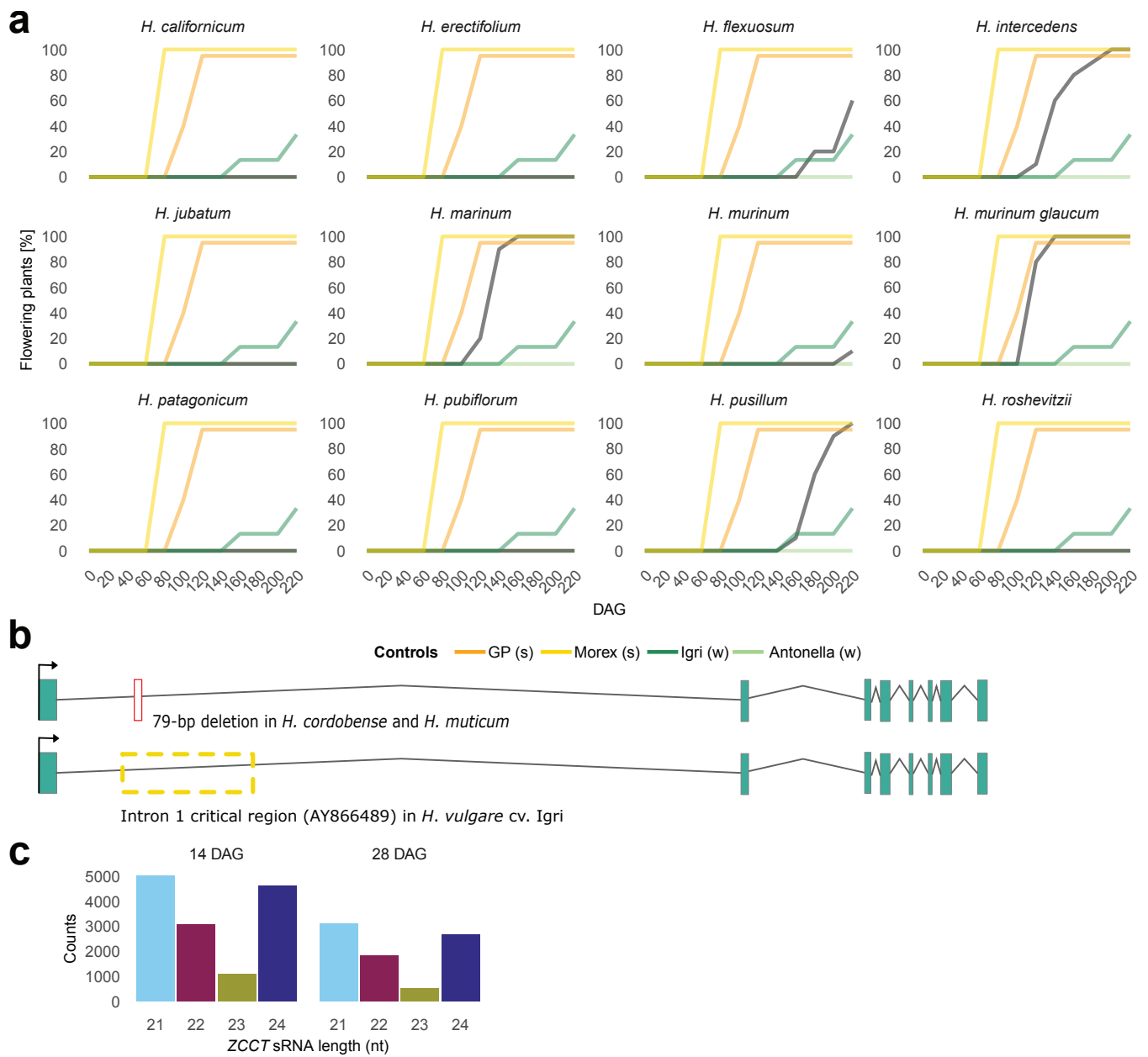
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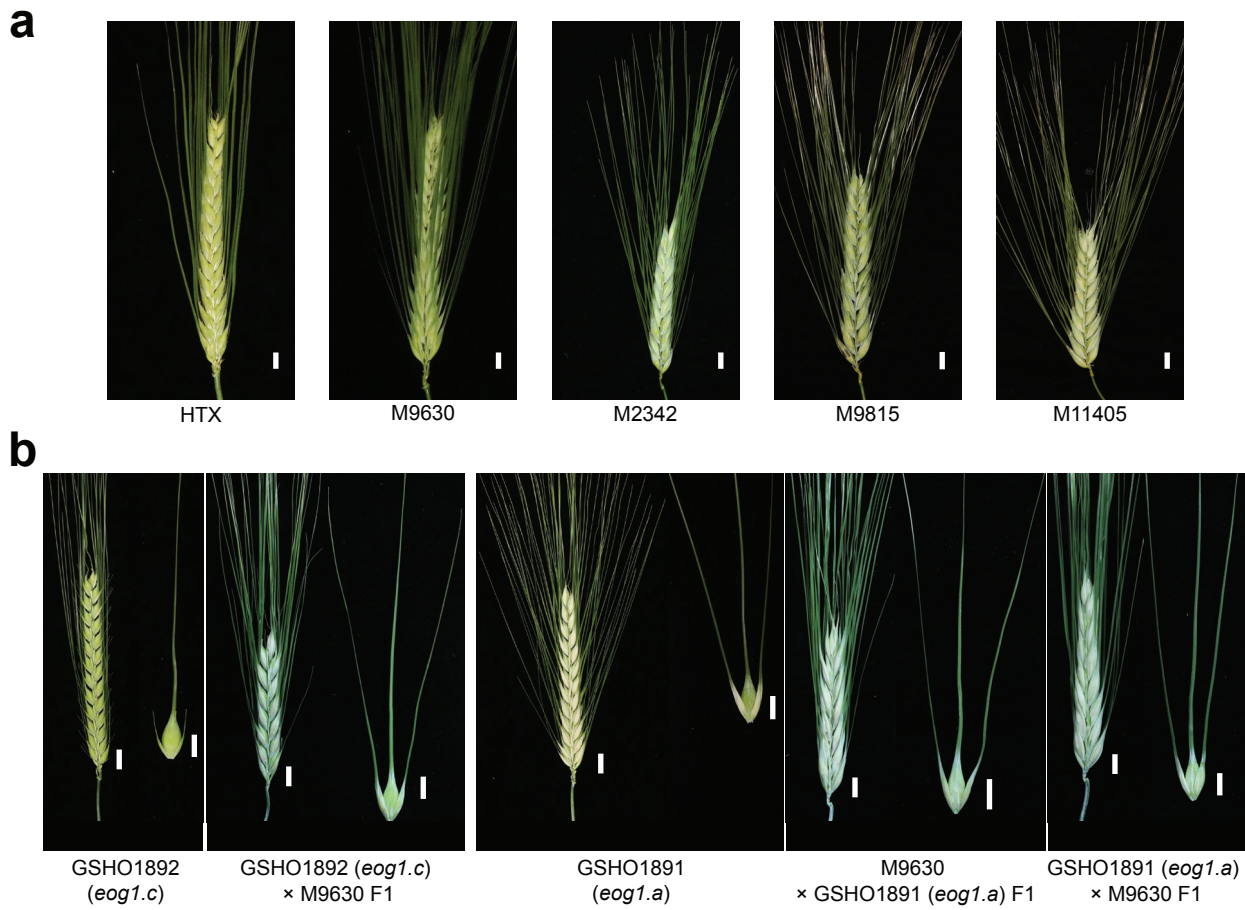
c



**Extended Data Fig. 8: Sequence conservation and divergence across *Hordeum*.** (a) Genome-wide sequence retention of functional elements along the phylogeny based on multiple sequence alignments (MSAs) of *Hordeum* species and 4 outgroup taxa (as shown in **Extended Data Fig. 7**). (b) Distribution of conserved sequences identified by PhastCons across 30 *Hordeum* genomes.haplomes. A y-axis value of 0.1 corresponds to 100 kb of conserved sequence per 1 Mb bin. (c) Enrichment of conserved elements in different functional categories; dot size indicates abundance.



**Extended Data Fig. 9: Flowering behavior and molecular basis of spring growth habit in wild *Hordeum*.** (a) Percentage of 12 wild *Hordeum* species plants flowering under non-vernalizing conditions. The cultivars Golden Promise (GP) and Morex versus cultivars Igri and Antonella serve as the spring-type (s) and winter-type (w) controls, respectively. DAG – days after germination. (b) Schematic representation of the *VRN1* gene. The 79 bp deletion present in the spring-type *H. cordobense* and *H. muticum* is outlined by a red rectangle and falls within the *VRN1* intron 1 critical region as defined in winter cultivar Igri (AY866489)<sup>50</sup>. (c) Size distribution of sRNAs mapped to *H. cordobense* ZCCT-2 and -3 genes at 14 and 28 DAG.



**Extended Data Fig. 10: Spike phenotype and test for allelism between M9630 and *eog1* *H. vulgare* mutants. (a)** Glume elongation phenotype on a spike. **(b)** Glume phenotype in F1 plants through crossing M9630 with other *eog1* mutants. Scale bar = 5 mm.