Supplementary Methods

Selection of genes for targeted capture assay

A set of genic and upstream regulatory sequences selected for enrichment comprised a comprehensive subset of loci related to flowering time and development of meristem and inflorescences. Additionally, a set genes related to agronomic traits putatively affected by domestication, e.g. tillering, seed dormancy, carbohydrate metabolism, was selected. First, scientific literature was mined for the genes implicated in the aforementioned processes and the corresponding nucleotide sequences were extracted from NCBI GenBank. Second, flowering genes from the other grass species, such as Brachypodium and rice, were selected (Higgins et al., 2010). Third, a set of 259 Arabidopsis genes characterized by the flowering-related gene ontology (GO) terms that have been confirmed experimentally was assembled (**Supplementary table 3**). The barley homologs of all these genes were extracted from the NCBI barley UniGene set (Hv cDNA, cv. Haruna Nijo, build 59) either by the BLASTN search (e-value < 1e-7) or, in the case of Arabidopsis genes, by searching the annotation table downloaded from the NCBI UniGene server (ftp://ftp.ncbi.nih.gov/repository/UniGene/Hordeum_vulgare). This table was further used to reciprocally extract additional Hv homologs based on the Arabidopsis gene identifiers. If the BLAST search failed to identify a reliable Hv homolog, the homologs were searched in the barley High and Low confidence genes (MLOC cDNA) (IBGSC, 2012) and in the HarvEST unigene assembly 35 (http://harvest.ucr.edu).

Open reading frames (ORF) of Hv cDNA were predicted using OrfPredictor guided by the BLASTX search against Arabidopsis TAIR 10 database (Min et al., 2005). The predicted ORFs were aligned to the genomic contigs of barley cultivars Morex, Bowman and Barke using the Spidey algorithm implemented in the NCBI toolkit. The ORFs of the selected sequences were categorized as complete or partial based on the presence or absence of putative start and stop codons. The complete complementary DNA (cDNA) were selected and, if the complete cDNA was absent, partial gDNA and cDNA were included in the dataset. For several genes with previously characterized intronic regions, e.g. predicted to contain regulatory elements, complete genomic DNA (gDNA) were selected. In case when only partial cDNA was available, chimeric sequences were assembled from the Hv, MLOC and HarvEST cDNA using SeqMan software (DNASTAR Lasergene®8 Core Suite, Madison, WI, USA). The selected sequences were cross-annotated with NCBI UniGene Hv and IBGSC MLOC identifiers using reciprocal BLASTN (e-value < 1e-05). In addition to the coding regions and introns, the selection contained sequences up to 3 kilobase pairs (Kbp) upstream of the predicted start codons, which presumably corresponded to regulatory promoter regions.

A set of 1000 additional HarvEST genes was randomly selected such that they had no homology to target genes as determined by BLASTN and were evenly spread over all barley linkage groups according to the GenomeZipper map (Mayer et al., 2011). The 100-bp stretches of each of these genes were included in the enrichment library.

The target sequences were filtered and tiled with 100-bp selection baits using Nimblegen proprietary algorithm and the library of baits was synthesized as a part of the SeqCap EZ enrichment kit (design name 130830_BARLEY_MVK_EZ_HX3; Roche NimbleGene, Madison, WI). Barcoded Illumina libraries were individually prepared, then enriched and sequenced in 24-sample pools at the Cologne Center for Genomics facilities following the standard protocols.

Mapping reference design

The genic sequences from a variety of barley genotypes were used to design the enrichment library to ensure that the longest ORF and promoter regions were selected. However, most advanced physical and genetics maps have been developed for the barley cultivar Morex. Since mapping information is essential for the downstream analyses, the so-called Morex genomic contigs were used as a mapping reference provided that they comprised the entire regions tiled by the baits (**Supplementary Table 4**) (IBGSC, 2012). If such contigs were not available, the genomic contigs of the barley genotypes Bowman and Barke or the templates that were used for the bait design were included in the mapping reference.

To identify the off-target enrichment regions, the Illumina reads from 10 randomly selected barley genotypes were mapped to the complete Morex genome reference set (IBGSC, 2012). All genomic contigs that had at least one read mapped to them were included in the mapping reference. The Morex contigs were masked with “N”s at the regions of > 100 bp that exhibited > 97% homology with the original capture targets.

Quality check, mapping and SNP calling pipeline

The quality parameters of the paired-end Illumina libraries were assessed using FastQC tool (v. 0.11.2; http://www.bioinformatics.babraham.ac.uk/projects/fastqc). After filtering out optical duplicates, resulting from a PCR amplification, using the CD-HIT-DUP software (v. 0.5) (Fu et al., 2012), the paired-end read files were merged and henceforth treated as a single-end dataset. Next, based on the FastQC results, the reads were trimmed from both ends to remove low quality sequencing data, filtered to remove the remaining adaptor sequences and low-complexity artifacts using the FASTX toolkit (v. 0.0.14; http://hannonlab.cshl.edu/fastx_toolkit). The sequencing errors in the dataset were corrected using the Bloom-filter tool Lighter with the conservative set of parameters: k-mer size 23, alpha 0.2, and maximum corrections per read 2 (Song et al., 2014). The reference file was indexed for the downstream processing using Burrows-Wheeler Aligner 0.5.9-r16 (BWA), SAMtools and Picard tools (http://broadinstitute.github.io/picard) (Li and Durbin, 2010; McKenna et al., 2010). The groomed read datasets were mapped onto the reference genome using BWA (modules 'aln' and 'samse') with the following stringency parameters: missing probability (-n) 0.05, maximum number of gaps (-o) 2, and gap extensions (-e) 12. Some of the reference loci were present in the form of cDNA and the gDNA-derived reads mapped onto such targets may generate false positive SNP calls at the intron-exon junctions. To alleviate this problem, the reads that mapped to cDNA-derived targets were extracted, additionally trimmed by 14 bp from each end and remapped following the described procedure. Reads that mapped to several locations were filtered out.

Calling variant (SNP) and invariant sites was performed for each sample library separately using the GATK UnifiedGenotyper walker with the default parameters except for the following flags: -pcr_error_rate 5.0E-02; -output_mode EMIT_ALL_CONFIDENT_SITES. The sites passing the following hard filters: biallelic, allele count (AC) 2 or 0, depth of coverage (DP) > 8, mapping quality (MQ) > 20, Fisher strand (FS) < 60, were selected using GATK SelectVariants walker. The individual VCF files were merged into a multi-sample file using the GATK CombineVariants walker. This pipeline was implemented in a series of bash scripts adapted for high-performance parallelized computation.

Supplementary Note 1

Characteristics of the enrichment assay

Of all the targets, 88% were selected in a form of cDNA and 85% comprised putative promoter regulatory regions > 100 bp. The target sequences were mined from various barley genomic and transcript databases and the predicted open reading frames (ORF) of 126 genes were longer than those of the MLOC genes currently used as a barley reference gene set (IBGSC, 2012). For 52 % of the genes the complete ORFs could be mapped to the IBGSC Morex contigs, whereas the rest of the ORFs were partially or completely absent from the IBGSC reference genome. These apparently represent either the genic regions not yet incorporated in the Morex reference genome or the unique allelic variants.

To attenuate effects of the biased selection of genes on the estimates of genetic diversity, we selected fragments of 1000 random genes spread over the barley chromosomes. The enrichment design baits tiled in total 2.42 Mbp of the barley gene space (**Supplementary Table 3**).

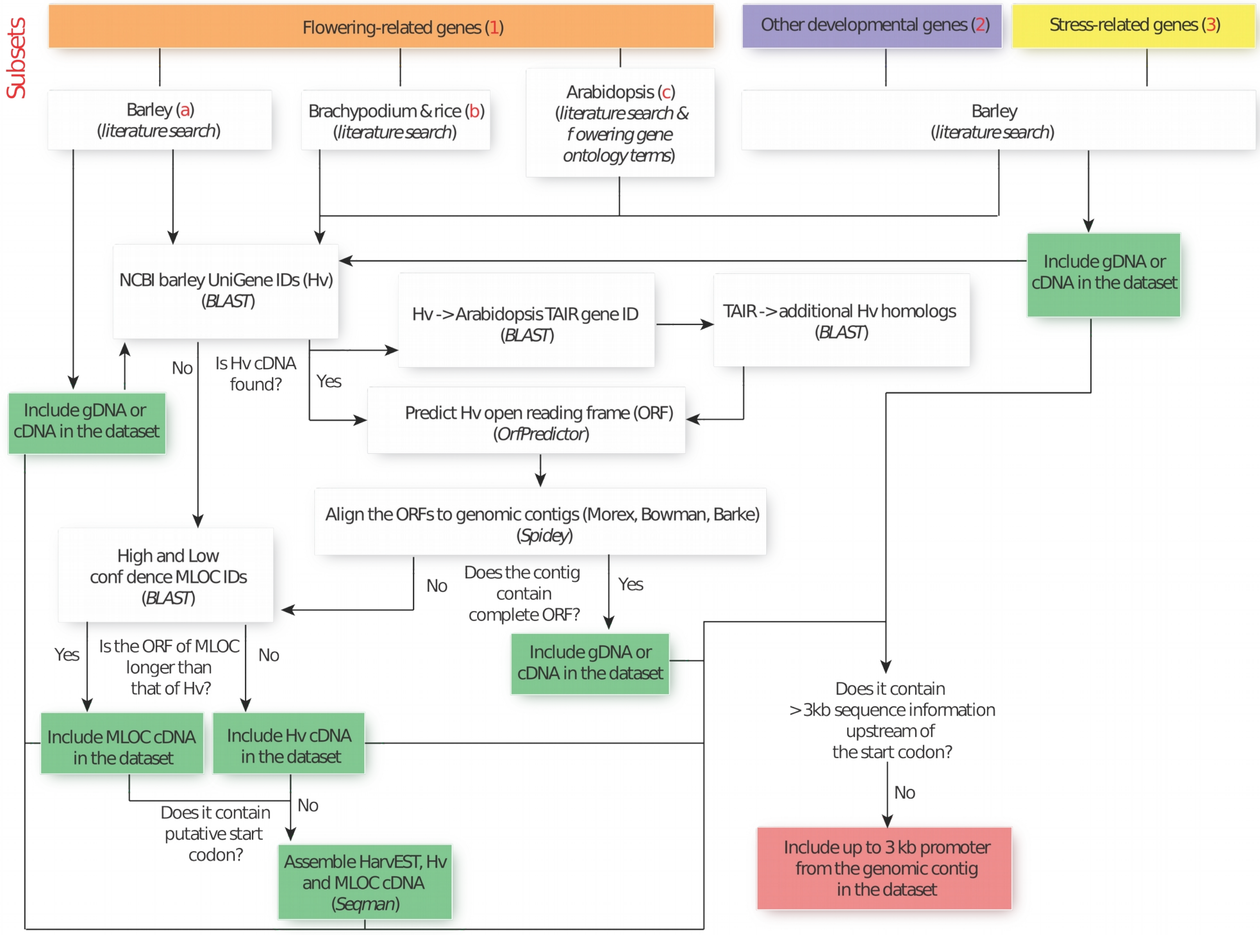
It has been shown that the hybridization-based enrichment assays, particularly NimbleGen SeqCap, are prone to generate off-target reads in the human exome capture assays (Bodi et al. 2013). In the human exome sequencing, large high-quality SNP datasets that originate from the off-target enrichment regions have been documented (Guo et al. 2012). Likewise, in this study, the size of the off-target captured regions was approximately six times larger than the size of the target capture design and yielded ~ 400.000 SNPs.

Supplementary Note 2

Wild barley population structure – a note of caution

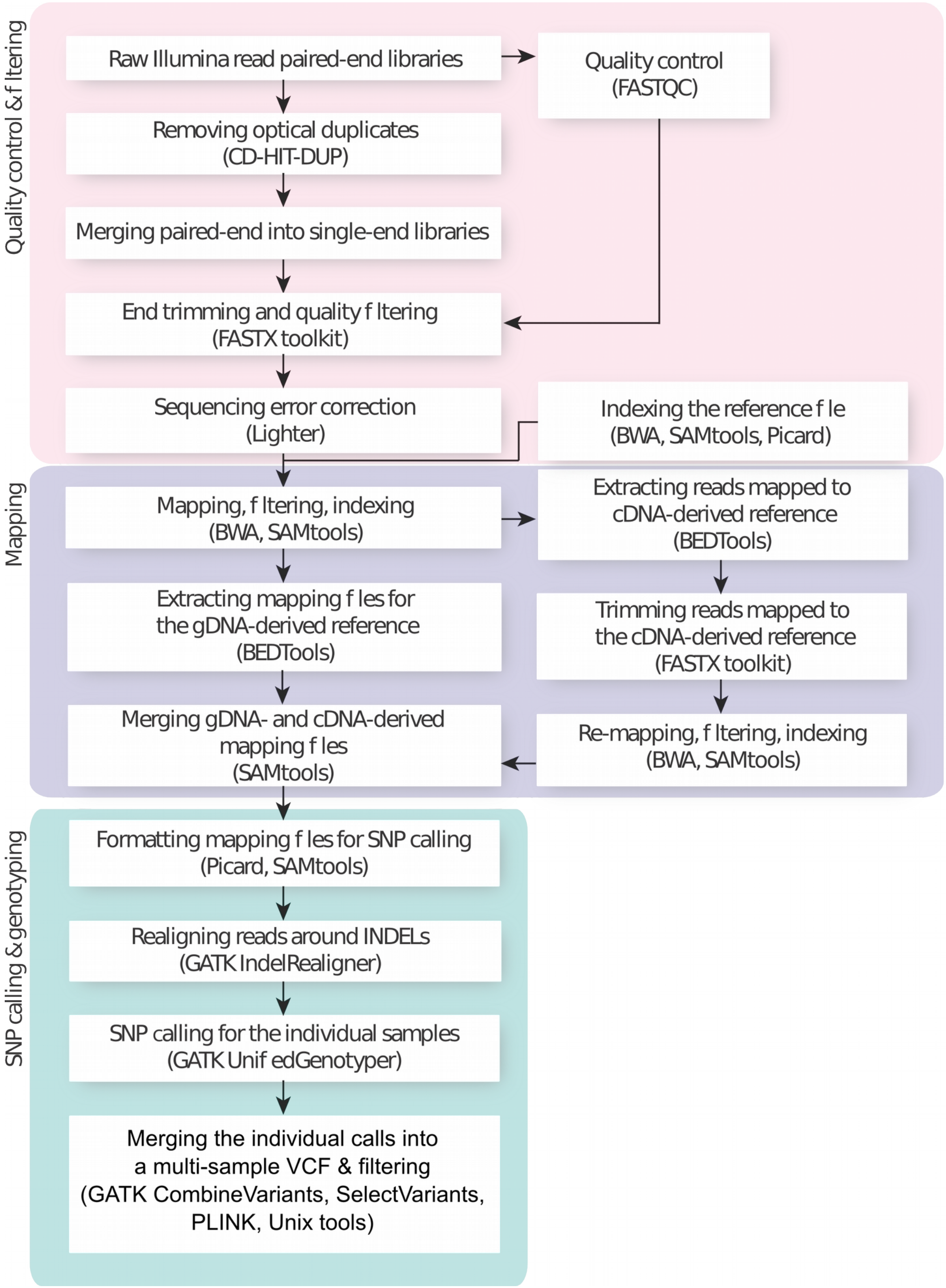
It is noteworthy that the output of the STRUCTURE models is not definitive and frequently a subject of misinterpretations (Falush et al. 2016). Here, both the phylogenetic and fastSTRUCTURE analyses strongly supported structuring genetic variation in wild barley into nine distinct clusters, which apparently represent subpopulations. However, we could not rule out that additional wild barley subpopulations may exist or that some of the genotypes detected as admixed in this study may, in fact, be non-admixed representatives of the undersampled populations. In future studies, sequencing of additional wild barley genotypes especially from the sparsely sampled regions – the Eastern horn of the Fertile Crescent - may help get further insights into the extent of these issues.

Supplementary Figures

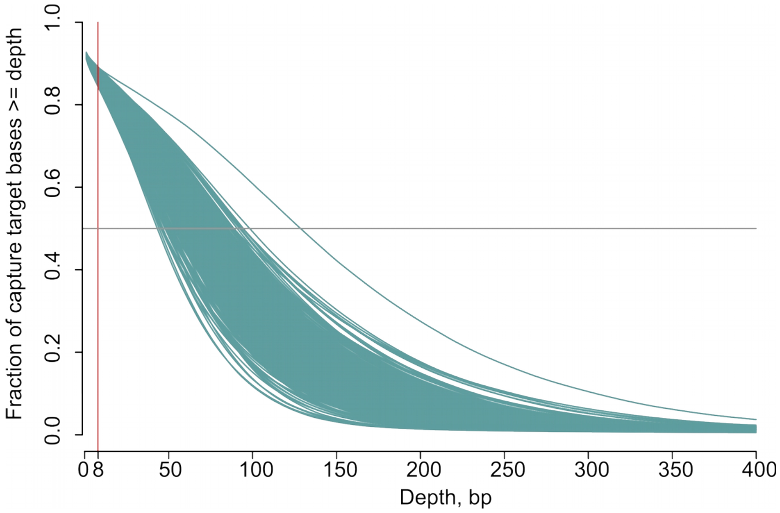

Subsets
Flowering-related genes (1)
Other developmental genes (2)
Stress-related genes (3)
Barley (a) (literature search)
Brachypodium & rice (b) (literature search)
Arabidopsis (c) (literature search & flowering gene ontology terms)
Barley (literature search)
NCBI barley UniGene IDs (Hv) (BLAST)
Hv -> Arabidopsis TAIR gene ID (BLAST)
TAIR -> additional Hv homologs (BLAST)
Include gDNA or cDNA in the dataset
Include gDNA or cDNA in the dataset
No
Is Hv cDNA found?
Yes
Predict Hv open reading frame (ORF) (OrfPredictor)
Align the ORFs to genomic contigs (Morex, Bowman, Barke) (Spidey)
High and Low confidence MLOC IDs (BLAST)
No
Does the contig contain complete ORF?
Yes
Include gDNA or cDNA in the dataset
Yes
Is the ORF of MLOC longer than that of Hv?
No
Include MLOC cDNA in the dataset
Include Hv cDNA in the dataset
Does it contain >3kb sequence information upstream of the start codon?
No
Include up to 3 kb promoter from the genomic contig in the dataset
Does it contain putative start codon?
No
Assemble HarvEST, Hv and MLOC cDNA (Seqman)

Supplementary Figure 1 Selection of target genes.

Subsets of three different functional categories of genes are highlighted in orange, violet and yellow. The output steps of the decision-making processes of selecting gene body sequences and promoter regions are highlighted in green and red, respectively.

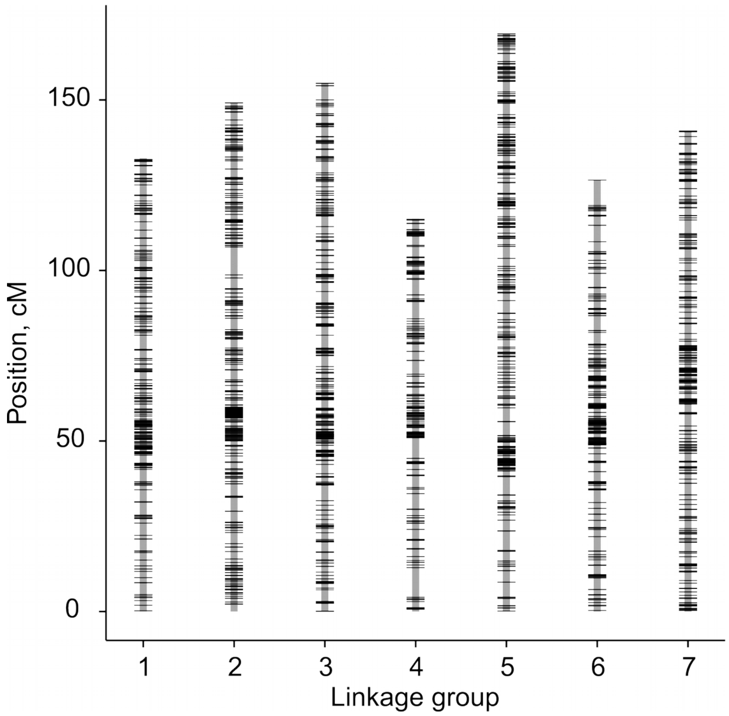

Quality control & f ltering
Raw Illumina read paired-end libraries
Quality control
(FASTQC)
Removing optical duplicates
(CD-HIT-DUP)
Merging paired-end into single-end libraries
End trimming and quality f ltering
(FASTX toolkit)
Sequencing error correction
(Lighter)
Indexing the reference f le
(BWA, SAMtools, Picard)
Mapping
Mapping, f ltering, indexing
(BWA, SAMtools)
Extracting reads mapped to
cDNA-derived reference
(BEDTools)
Extracting mapping f les for
the gDNA-derived reference
(BEDTools)
Trimming reads mapped to
the cDNA-derived reference
(FASTX toolkit)
Merging gDNA- and cDNA-derived
mapping f les
(SAMtools)
Re-mapping, f ltering, indexing
(BWA, SAMtools)
SNP calling & genotyping
Formatting mapping f les for SNP calling
(Picard, SAMtools)
Realigning reads around INDELs
(GATK IndelRealigner)
SNP calling for the individual samples
(GATK Unif edGenotyper)
Merging the individual calls into
a multi-sample VCF & filtering
(GATK CombineVariants, SelectVariants,
PLINK, Unix tools)

Supplementary Figure 2 The data analysis pipeline - read filtering, mapping, SNP calling and genotyping.

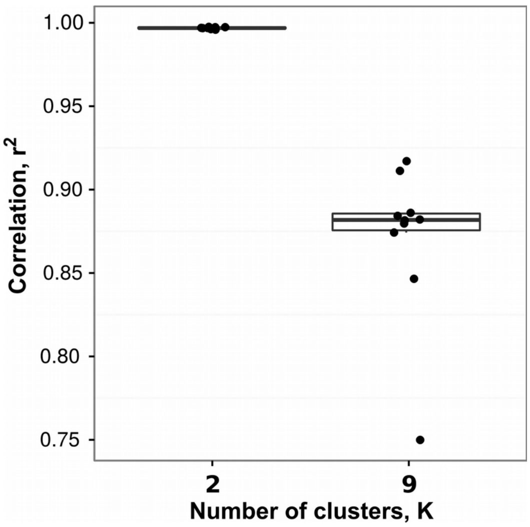


Supplementary Figure 3 Characteristics of coverage and polymorphisms.

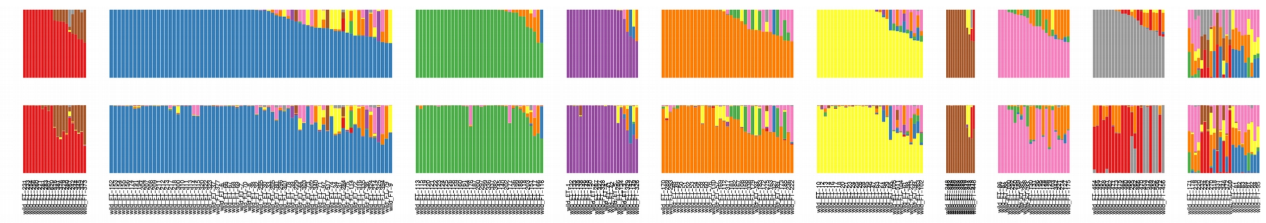
(**a**) A fraction of target nucleotides covered at a certain depth in the individual samples shown as cyan curves. A cut-off coverage threshold for the SNP calling and the median coverage are shown as vertical red and horizontal gray lines, respectively.



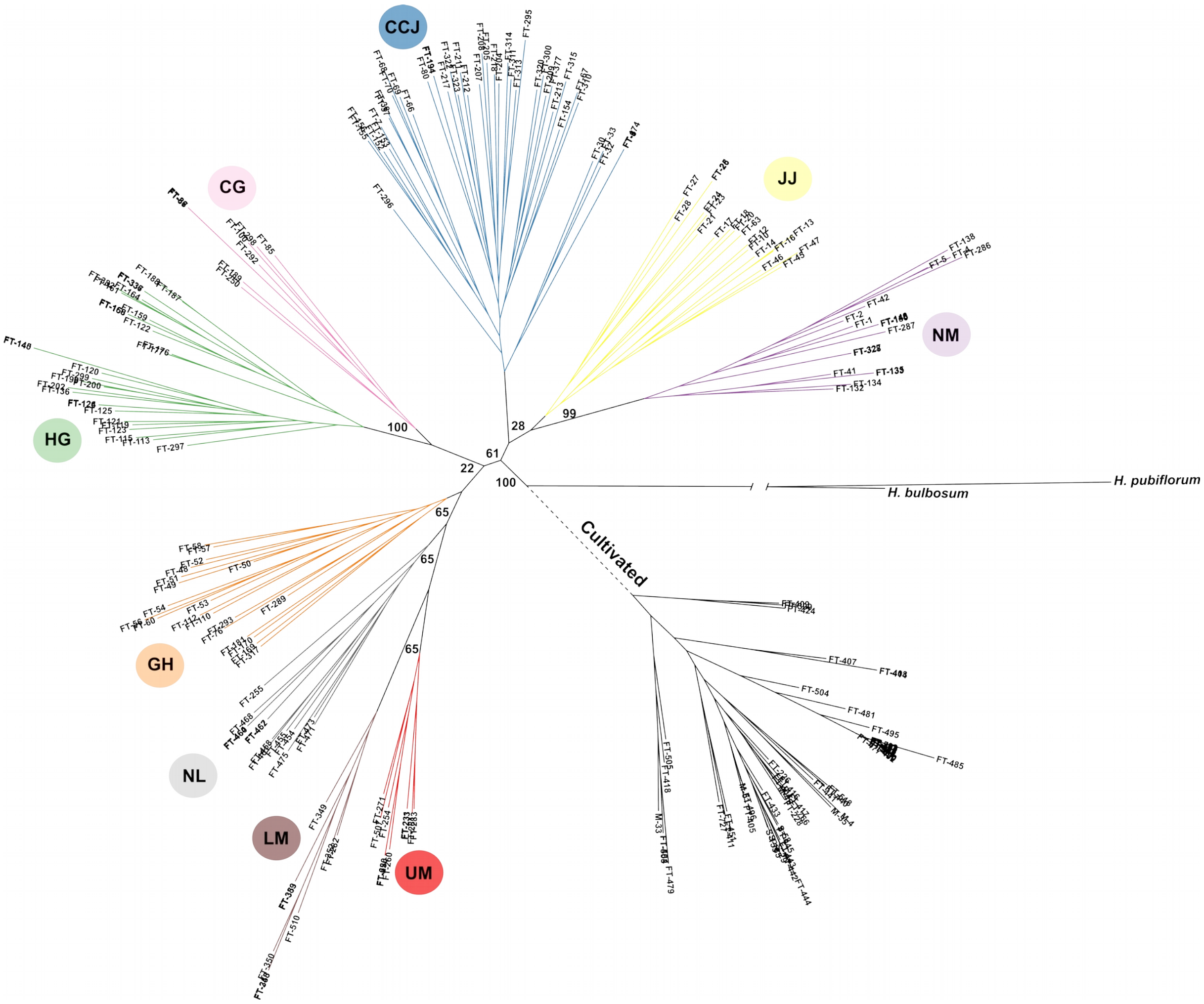
Supplementary Figure 4 Distribution of SNP markers over the barley chromosomes and transition / transversion (Ti / Tv) ratio. Mapping location of the SNP markers on barley linkage group based on the PopSeq map (Mascher et al., 2013). The linkage groups and marker positions are shown as vertical gray and horizontal black bars, respectively.



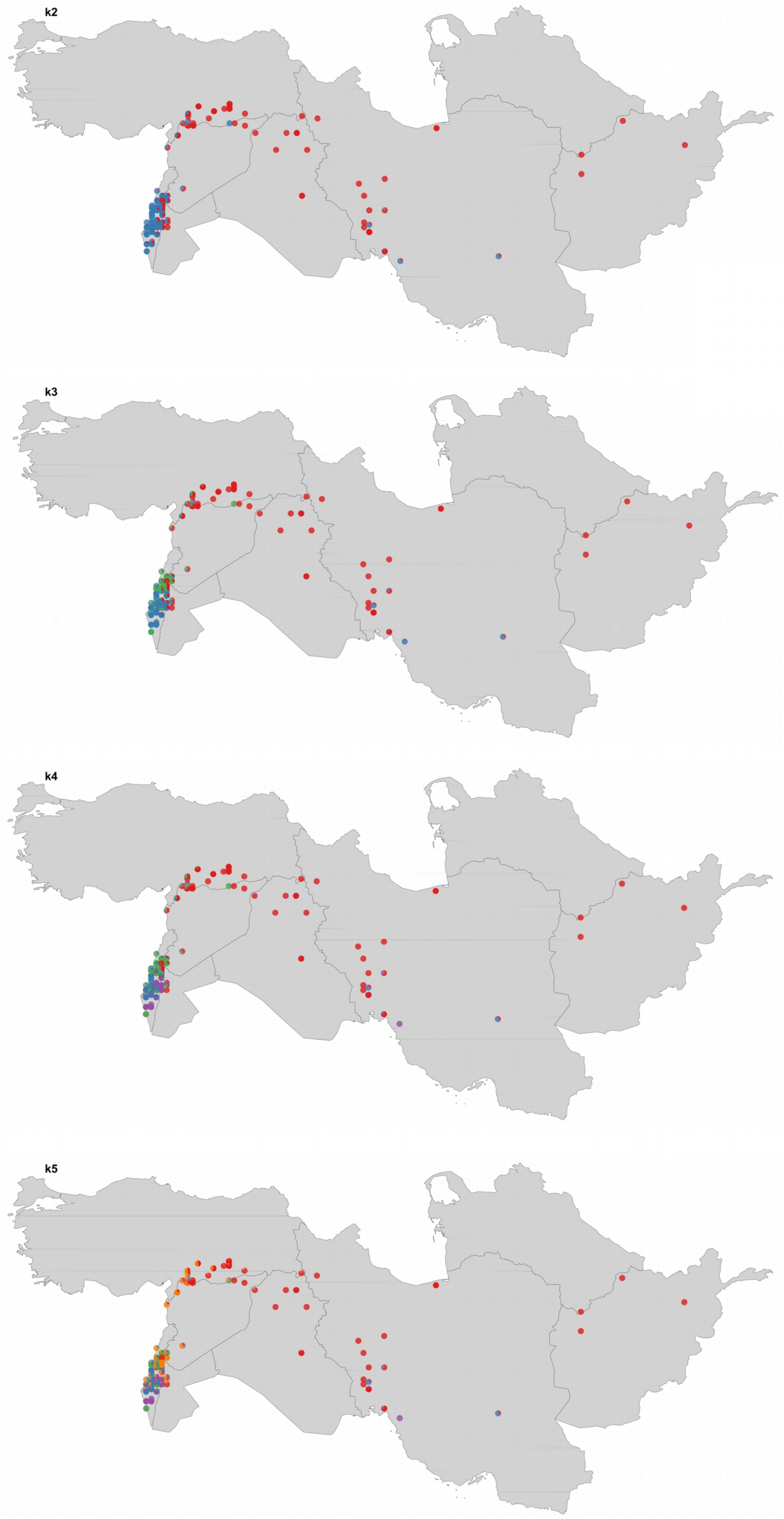
Supplementary Figure 5 Correlation of the ancestry coefficients estimated using fastSTRUCTURE and INSTRUCT models for the number of clusters K=2 (wild and domesticated) and 9 (wild).



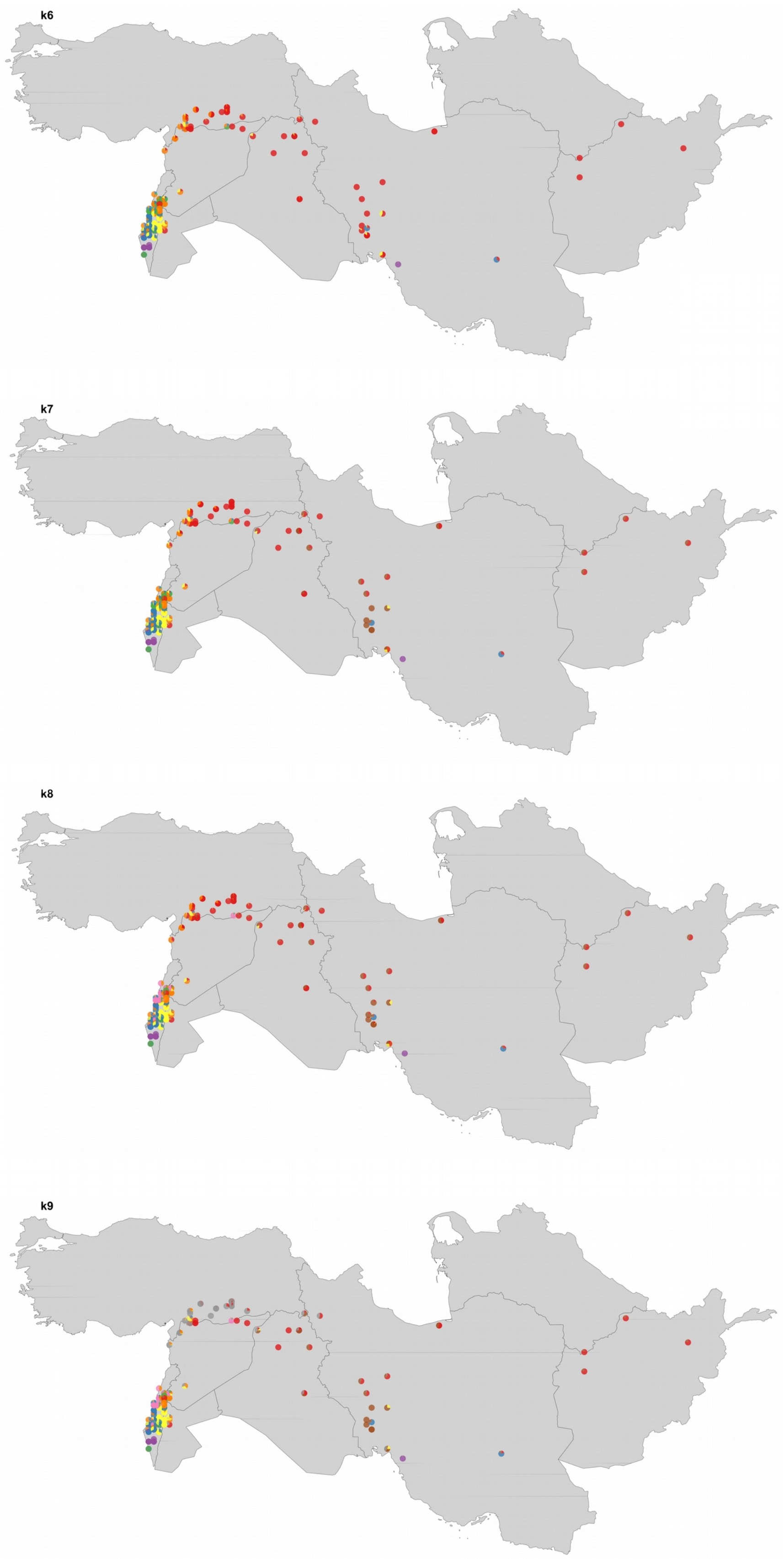
Supplementary Figure 6 Population structure of wild barley (K=9) as determined by fastSTRUCTURE and INSTRUCT models – upper and lower panels, respectively. Vertical bars correspond to individual genotypes and colors indicate their membership in the nine subpopulations.



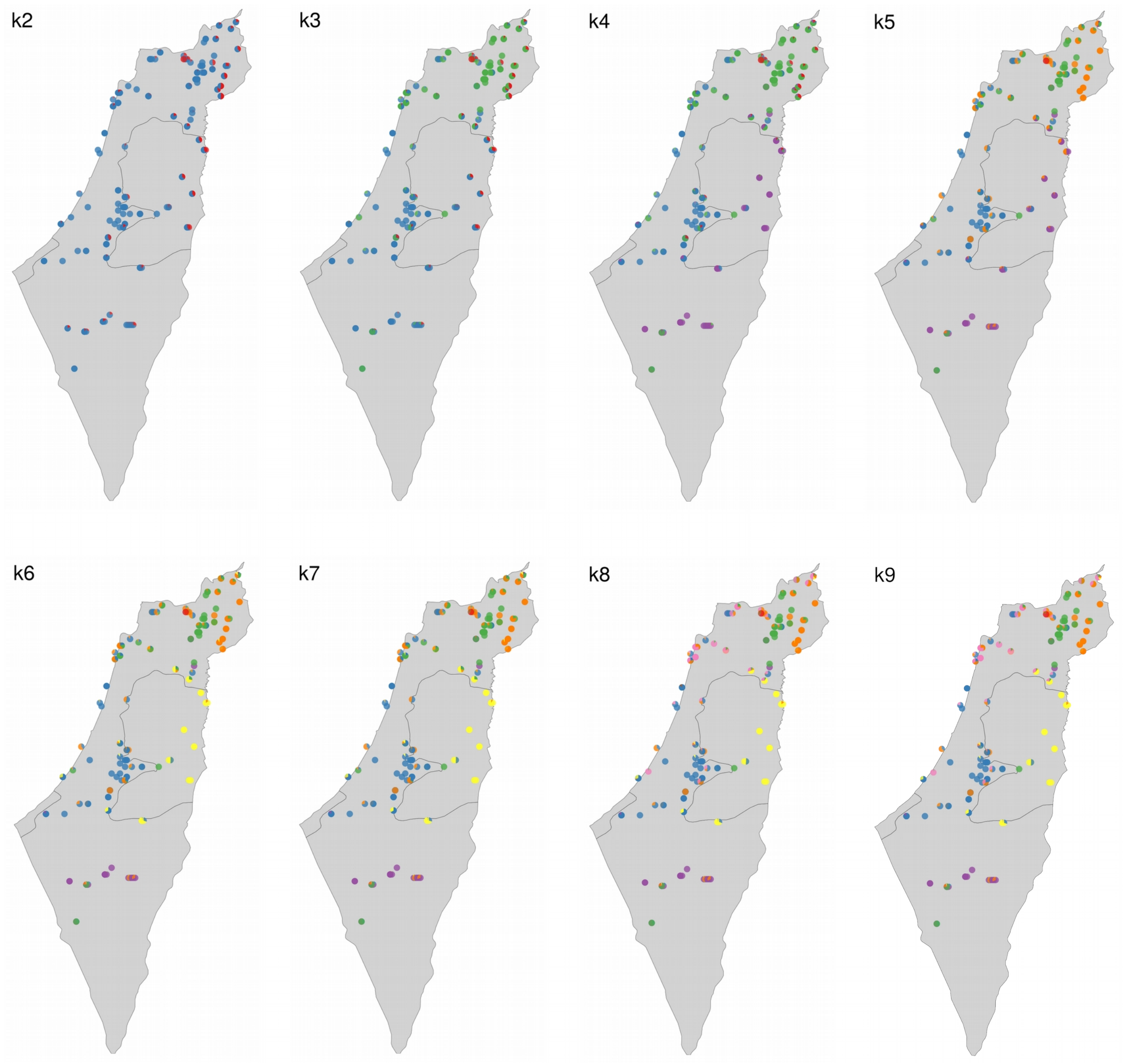
Supplementary Figure 7 The Maximum Likelihood (ML) unrooted phylogeny of 230 barley accessions. Coloured clusters correspond to the nine wild barley (H. vulgare ssp. spontaneum) populations. Carmel & Galilee (CG; pink); Golan Heights (GH; orange); Hula Valley & Galilee (HG; green); Judean Desert & Jordan Valley (JJ; yellow); Lower Mesopotamia (LM; brown); Negev Mountains (NM; magenta); North Levant (NL; grey); Sharon, Coastal Plain & Judean Lowlands (SCJ; blue); Upper Mesopotamia (UM; red). Cultivated barley (H. vulgare ssp. vulgare) is shown as a black cluster. The dashed line indicates that the phylogenetic placement of the cultivated barley cluster may be uncertain due to its complex hybrid origin. Wild barley *H. bulbosum* and *H. pubiflorum* are used as distant outgroup species and the length of the outgroup branch is artificially shortened. The bootstrap values are shown at the corresponding nodes.



Supplementary Figure 8 Distribution of the wild barley populations within the Fertile Crescent. The pie charts, reflecting ancestral composition of the individual genotypes as determined by fastSTRUCTURE for K from 2 to 9, are shown at geographic location of the genotypes. See Supplementary Fig. 7 for the color legend.

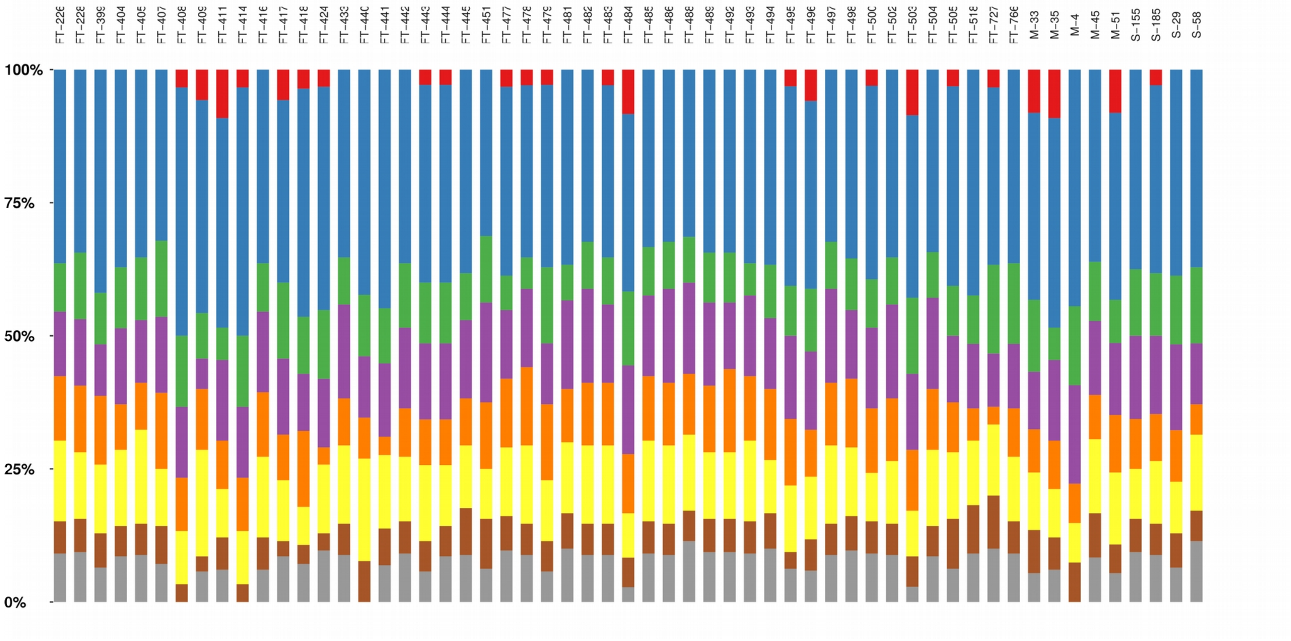


Supplementary Figure 8 *(continued)* Distribution of the wild barley populations within the Fertile Crescent. The pie charts, reflecting ancestral composition of the individual genotypes as determined by fastSTRUCTURE for K from 2 to 9, are shown at geographic location of the genotypes. See Supplementary Fig. 7 for the color legend.

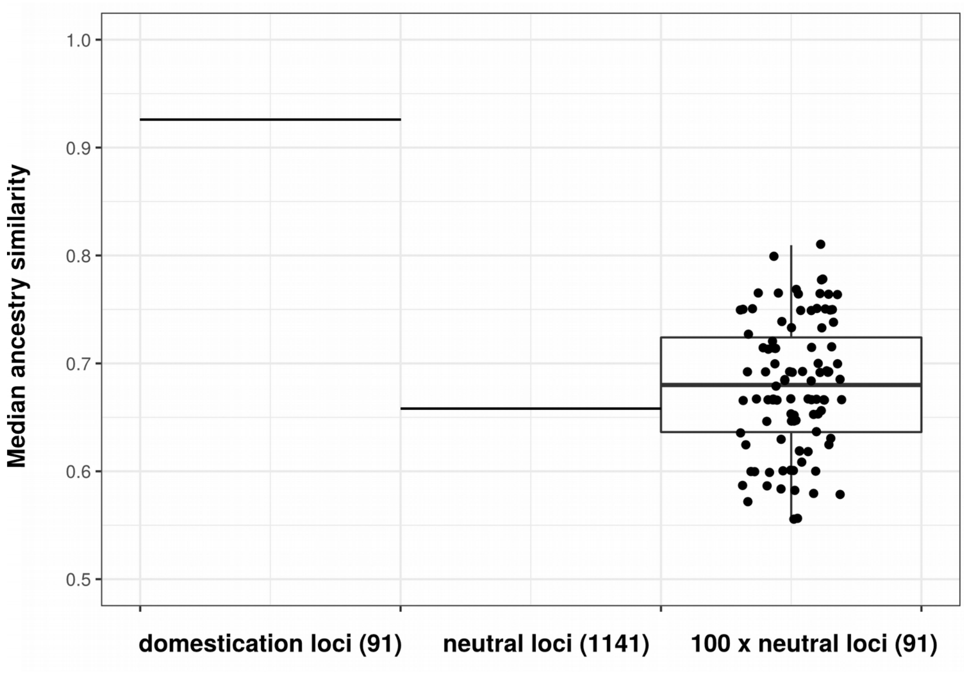


Supplementary Figure 9 Distribution of the wild barley populations within Israel and Palestine.

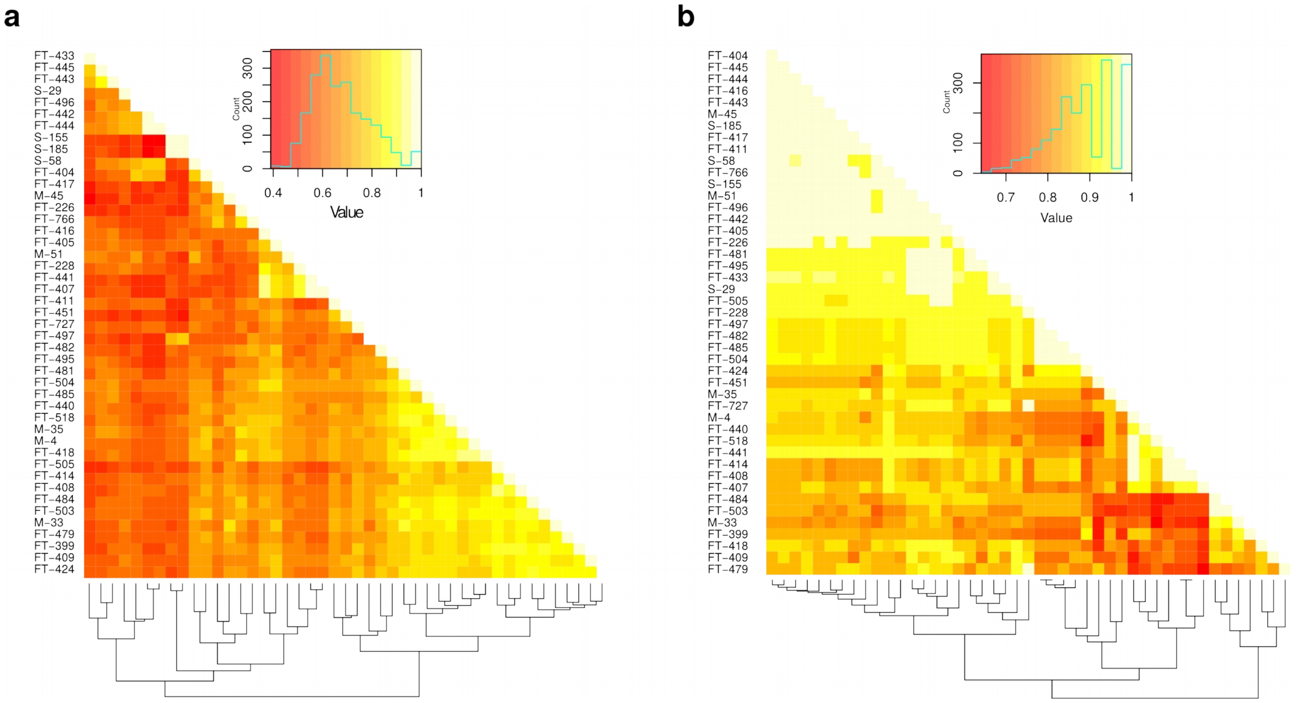
The pie charts, reflecting ancestral composition of the individual genotypes as determined by fastSTRUCTURE for K from 2 to 9, are connected to their geographic location. See Supplementary Fig. 7 for the color legend.



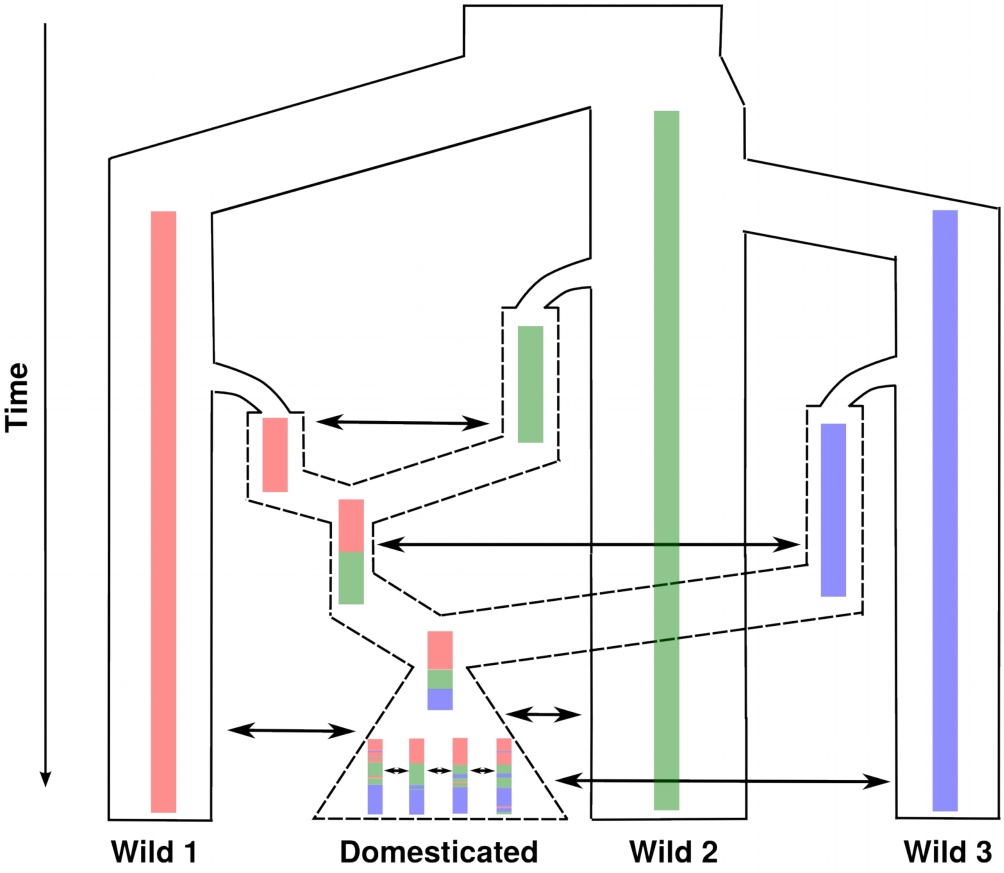
Supplementary Figure 10 Ancestral palettes of the candidate domestication loci. See Supplementary Fig. 7 for the color legend.



Supplementary Figure 11 Estimation of the median ancestry coefficients in the unbalanced subgroups of loci (91 domestication and 1141 neutral loci) and in the 100 randomly drawn subsets of 91 neutral loci.



Supplementary Figure 12 Heatmaps of the pairwise ancestry similarity coefficients. The insets represent the color legend and contain the histograms of the ancestry similarity coefficients calculated for the neutral (**a**) and domestication sweep loci (**b**).



Supplementary Figure 13 A simplified hypothetical demographic model of multiple barley domestications proposed based on the ancestry patterns of the domesticated barley genomes. Red, green and blue colors represent three founder populations of wild barley (solid lines) and corresponding independent domestication lineages (dashed lines). The colored bars are analogous to the ancestry palettes. The double-sided arrows illustrate gene flow between the lineages.

Supplementary Tables

Supplementary Table 5 Characteristics of the enrichment assay and SNP calling.

	Selected size, Mbp	**Captured, Mbp**	**Captured CDS, Mbp**	**Homozygous SNPs**		
				Total, with singletons	Total, w/o singletons	Filtered set*
Target	2.42	2.24	0.85	121,294	83,752	20,954
Off-target	-	11.56	0.48	423,024	270,858	34,682
Total	9.91	13.80	1.33	544,318	354,610	55,636

* - minor allele frequency < 0.05; missing data frequency < 0.5

Supplementary References

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