A haplotype-resolved pangenome of the barley wild relative Hordeum bulbosum

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Abstract

Wild plants can contribute valuable genes to their domesticated relatives. Fertility barriers and a lack of genomic resources have hindered the effective use of crop-wild introgressions. Decades of research into barley's closest wild relative, *Hordeum bulbosum*, a grass native to the Mediterranean basin and Western Asia, have yet to manifest themselves in the release of a cultivar bearing alien genes. Here, we construct a pangenome of bulbous barley comprising ten haplotype-resolved genome sequence assemblies. Autotetraploid cytotypes, among which the donors of resistance-conferring introgressions are found, arose at least twice, and are connected among each other and to diploid forms through gene flow. The differential amplification of transposable elements after barley and *H. bulbosum* diverged from each other is responsible for genome size differences between them. We illustrate the translational value of our resource by mapping non-host resistance to a viral pathogen to a structurally diverse multigene cluster implicated in diverse immune responses in wheat and barley.

Full Text

Wild relatives of cultivated plants are important sources of genetic diversity for crop improvement[1]. A common means of transferring genes from wild into domesticated plants are introgression lines that are derived from crop-wild crosses and bear a small proportion of the wild parent's genes in an otherwise cultivated genomic background. Developing these "alien" introgressions into elite varieties is challenging in many crops. A case in point is barley (*Hordeum vulgare* L.). To begin with, barley's "secondary gene pool[2]" – those wild species that are amenable to gene transfer via meiotic recombination – is narrower than that of wheat, another grain crop and fellow member of the Triticeae tribe of cereal grasses, in which introgression lines have been more widely deployed[3,4]. There are more than 30 species in the genus *Hordeum*[5], all of which might be considered candidate donors of valuable alleles. Yet only one yields fertile progeny when crossed with barley: bulbous barley (*Hordeum bulbosum* L.). Although *H. bulbosum* is also barley's closest wild relative with an estimated divergence time of 4.5 million years between the two species[5], their genetics are quite different. Bulbous barley is a perennial outcrosser with either a diploid or an autotetraploid genome[6]. By contrast, barley breeding and research have much profited from the tractable genetics of a diploid annual inbreeder. It is not for lack of trying that crop-wild introgressions have yet to meet with agronomic success in barley. Wide-crosses yielded introgression lines in which the distal regions of any single chromosome have been replaced with their homologs in bulbous barley[7,8]. Resistances to numerous diseases are common in this germplasm, but so are yield penalties[9]. This is because of linkage drag, the co-transfer with beneficial alleles of harmful variants. To get rid of the latter, geneticists and breeders have to characterize introgression lines with molecular markers and select rare recombinants to fine-map resistance loci[10,11]. Genomic tools developed in barley have helped in these efforts[9,12], but owing to structural variation and differences in gene content barley is an imperfect proxy at best. Thanks to accurate long-read sequencing[13], pangenomes, collections of genome sequences of multiple individuals of a species or higher taxon[14], are taking over the role of

single reference genome as anchors for genetic mapping and comparative genomics. Here, we report a haplotype-resolved pangenome of *H. bulbosum* and illustrate its applications in evolutionary research and trait mapping.

A diploid reference for H. bulbosum

We first attempted the sequence assembly of a diploid *H. bulbosum* clone, FB19-011-3, grown from seed collected close to Andirrio in western Greece. The contiguity and completeness of the contigs constructed from PacBio HiFi reads[13] (**Supplementary Table 1 and 2)** were promising: the assembled contigs amounted to 6.78 Gb of sequence and half of them were longer than 9.82 Mb. This level of contiguity is comparable to those achieved in inbred wheat[15] and barley[16]. The good separation between the haplotypes is reflected by a preponderance of unique 71-mers over duplicated ones in *k*-mer profiles of the HiFi reads (**Supplementary Fig. 1**). Chromosome-conformation capture sequencing (Hi-C) data[17] was used to partition contigs to haplotypes **(Extended Data Fig. 1a, Methods)**, which were then arranged into chromosomal pseudomolecules with the help of physical linkage information provided by Hi-C data and a Bionano genome map (**Supplementary Table 3, 4 and 5**). The resultant pseudomolecules were longer than their constituent contigs because there was a large identity-by-descent (IBD) tract spanning the centromere of chromosome 4H (**Extended Data Fig. 1b**). Both haplotypes were highly colinear to the barley genome (Spearman's rank correlation: 0.9991, **Fig. 1a**). This was expected as gene order is conserved even between barley and the D subgenome of wheat, which diverged 8-9 Mya from each other[18]. Visual inspection of the Hi-C contact matrices supported the absence of phase switches or other gross misassembles (**Fig. 1b**). The genome assembly was shorter than expected from flow-cytometric estimates (7.37 Gb vs. 8.68 Gb)[19], but was 80 % as long as that of *H. vulgare*, irrespective of whether assembly (8.46 Gb) or flow-cytometry (10.36 Gb) was consulted. We have attributed this discrepancy in barley to unresolved repeat arrays in ribosomal loci and centromeric and interspersed satellites[20].

To obtain further support for the structural integrity of our assembly, we genotyped single-pollen nuclei of FB19-011-3 through Illumina short-read sequencing[21] (**Fig. 1c, Supplementary Table 6**), aligned the reads to one of the haplotypes and called single-nucleotide polymorphisms (SNPs). In the resultant variant matrix, we looked for patterns consistent with the presence of phase switches and found fewer than 50% SNPs (1.01%) that were potentially located in wrongly phased regions (**Extended Data Fig. 1c).** The genetic map constructed from those data was colinear with the genome sequence (**Fig. 1d**). Pericentric recombination deserts common to all Triticeae extended further into distal regions in *H. bulbosum* than in barley (**Fig. 1d**). This is borne out by cytological observations: extra-terminal chiasmata, i.e. those where homologous chromosomes were distally connected only by a thin thread of chromatin during meiotic metaphase I, were twice as frequent in *H. bulbosum* as in barley (**Extended Data Fig. 2**). To gain more confidence in the accuracy of our haplotype phasing, we designed oligonucleotide probes[22] for single-copy regions on the long arm of chromosome 5H that are unique to either haplotype (**Extended Data Fig. 3**). Fluorescence *in situ* hybridization (FISH) showed the expected

red and green signals marking on both 5H homologs without interspersed or mixed signals (**Fig. 1e, Extended Data Fig. 4a**). Hybridization of the probe set to meiotic chromosomes of FB19-011-3 and mitotic chromosomes of selfed offspring also allowed us to visualize cross-overs events (**Extended Data Fig. 4)**. Taken together, collinearity to barley, Hi-C signals, genetic mapping and FISH supported the accuracy of the haplotype-resolved assembly of FB19-011-3. A total of 70,308 high-confidence gene models were annotated on the genome sequence with evidence from homology, Illumina RNA sequencing and PacBio isoform sequencing data (**Supplementary Table 7**). BUSCO, a commonly used benchmark of gene space completeness[23], indicated that 98.3 % of universal single-copy orthologs were present in both haplotypes (**Supplementary Table 8**). The long terminal repeat (LTR) Assembly Index (LAI), which evaluates the contiguity of intergenic and repetitive regions of genome assemblies based on the intactness of LTR retrotransposons, of the FB19-011-3 genome assembly was 20.52, similar to that of barley cv. Morex (14.72) (**Supplementary Table 8)**.

A haplotype-resolved pangenome of H. bulbosum

A pangenome of bulbous barley would be incomplete if it did not include tetraploid genotypes. We had phased a diploid sequence assembly essentially by a principal component analysis on the Hi-C data and expected that this approach should work for higher ploidies as well. We attempted the assembly of a tetraploid clone, FB19-023-3 (**Extended Data Fig. 1d and Methods)**. Contig-level contiguity was lower than in the diploid, but still half of the assembled sequence was in contigs longer than 7.7 Mb. IBD haplotypes, but no misassemblies, were discernible in Hi-C contact matrices (**Fig. 2a)**. No phase switches were seen in four-color FISH with haplotype-specific painting probes for the long arm of chromosome 5H (**Fig. 2b, Extended Data Fig. 3c**). Having convinced ourselves of the feasibility of haplotype-resolved genome assembly, we selected another three diploid and five tetraploid lineages for genome sequencing to arrive at a total of 32 haplotypes (**Supplementary Fig. 2-9and Supplementary Table 9**). The selected genotypes span the geographic range of the species from Central Asia to the Western Mediterranean (**Fig. 2c)**. Among them are also three genotypes, A17, A40, A42, that have served as donors to introgression lines. The assembly metrics of diploid and tetraploid genotypes were comparable: initial unphased assemblies amounted to between 6.9 Gb and 14.2 Gb and had N50 values between 6.4 Mb and 17.0 Mb (**Supplementary Table 8**). In the reads of all genomes, unique 71-mer dominated k-mer profiles, but IBD between haplotypes was more pronounced in some genomes than in others (**Supplementary Fig. 1**). Locally high similarity or pairwise identity of haplotypes was accompanied, respectively, by lower assembly contiguity or fewer than otherwise the four haplotypes. Nevertheless, the Hi-C contact matrices enabled us to assign the contigs of the primary assemblies to one or more haplotypes (**Extended Data Fig. 5**). Diploid assemblies were more complete and contiguous than their tetraploid counterparts, but the latter still met the quality standards of the EarthBiogenome project[24]. The pseudomolecules of the diploid assemblies had an average size of 7.4 Gb. The corresponding number for the tetraploid was 14.3 Gb. BUSCO scores were between 98.0% and 98.9% and LAI scores ranged from 15.41 to 16.14 (**Supplementary Table 8**). Between 88.1 % and 97.7 % of the contigs of the primary assembly were incorporated into pseudomolecules and partitioned into

haplotypes, whose size ranged from 3.0 to 3.6 Gb **(Supplementary Table 9)**. Additionally, ten complete chloroplast genomes, with an average genome size of 136,680 base pairs, were assembled **(Supplementary Table 8)**. Gene models were annotated by projecting the FB19-011-3 models to the other genome sequences (**Supplementary Table 8 and 9**). Gene order in the 32 haplotypes was conserved. Large (> 2 Mb) inversions were evident from the whole-genome alignments, but we did not observe any interchromosomal translocations, consistent with the evolutionary stability of the Triticeae's seven-chromosome karyotype (**Fig. 2d**). The largest event we detected was a 243 Mb inversion private to haplotype of FB19-028-3 (**Supplementary Data Figure 6a**). Distal inversions between the haplotype of FB19-011-3 corresponded to regions of suppressed recombination as revealed by pollen sequencing (**Supplementary Data Figure 6b and c**). The distributions of SNPs as well as insertions and deletions followed a centrifugal gradient (**Extended Data Figure 6d**), as do many genomic features in the Triticeae[25,26]. A reference-free assessment of sequence diversity based on *k*-mers revealed higher diversity in *H. bulbosum* than in *H. vulgare* subsp. *spontaneum*, the wild progenitor of domesticated barley (**Fig. 2e**). A graph constructed from single-copy sequences[27] in the *H. bulbosum* pangenome grew faster in complexity than its correspondent in barley (**Fig. 2f**). The greater diversity of *H. bulbosum* reinforces the notion that it is an untapped reservoir of diversity for its domesticated relatives.

TEs underlie genome size differences

Studies of structural evolution in the repeat-rich genomes of *H. bulbosum* and *H. vulgare* cannot limit themselves to genes and other low-copy sequences. We annotated transposable elements (TEs) and other classes of repetitive elements in both our *H. bulbosum* assemblies and a long-read assembly of the barley genome with the EDTA pipeline[28] using the TREP database[29]. TEs make up 80-90 % of the barley genome, as they do in *H. bulbosum* (**Extended Data Fig. 7a and Supplementary Table 10**). The genome of *H. bulbosum* is about 80 % the size of the barley genome and much of this is attributable to a difference in overall TE content (3.70 Gb in barley cv. Morex, 3.14 Gb in *H. bulbosum* FB19-011-3). The predominant class of TEs in both genomes are LTR retrotransposons of the *Copia* and *Gypsy* superfamilies (**Fig. 3a**). But TE profiles differ locally as do the relative rates of genome size expansion differ as revealed by a generalized additive model fitted to chromosome-scale alignments (**Fig. 3b and Supplementary Fig.10**). Despite their overall smaller size, distal regions of the *H. bulbosum* genome are relatively longer than those of *H. vulgare* (**Fig. 3c**), mainly because *Gypsy* elements inserted there more often in the former species. The expansion of *H. vulgare* genome, by contrast, is not attributable to any single class of TE in proximal regions (**Fig. 3d**). Since those TE bursts that are still discernible in today's genomes all happened after the divergence of barley and *H. bulbosum* (**Fig. 3e**), sequences conserved between both genomes are limited mainly to genes and their regulatory sequences. The same TE families have colonized the genomes of both species, but which of their members inserted where and when sets the species apart (**Extended Data Figure 7b, c**). BARE-1 elements, a particularly well-studied family of *Copia* LTR retrotransposons[30], make up 493 Mb of the Morex genome, while its closest homologs in FB19-011-3 account for only 302 Mb of sequence (**Extended Data Fig. 7c, d**). Vice versa, Sabrina, a family of *Gypsy* elements, amounts to 501 Mb in *H.*

bulbosum, but only 420 Mb in *H. vulgare* (**Extended Data Fig. 7c)**. These differences in overall sequence content are reflected by those in the timing and intensities of bursts of BARE-1 and Sabrina (**Extended Data Fig. 7e and f**). Although smaller in size, the genome of *H. bulbosum* has more full-length LTR elements (61,916) than that of *H. vulgare* (46,727). This is in line with the TE insertion in the last 300,000 years, mainly of Gypsy elements in proximal regions (**Fig. 3e and f**). These relative differences between the two genomes should not overshadow the fact that in both species TE insertions happened most frequently in distal regions, where young elements predominate (**Fig. 3f and Extended Data Fig. 7g**).

Autotetraploids arose at least twice

H. bulbosum is curious in that this taxon is composed of both tetraploid and diploid members. It is expected that the former trace back to the latter and that both groups are reproductively isolated. Yet, despite different chromosome numbers, genome sizes and clear genetic differentiation (**Fig. 4a and b, Extended Data Fig. 8a**), diploid and tetraploid plants are morphologically indistinguishable[6]. Pertinent questions are: how often have tetraploids arisen, how are they related to diploids, and is there gene flow between both groups? To answer these questions, we studied the divergence of our 32 pangenome haplotype and 263 lineages genotyped by reduced representation sequencing (**Extended Data Fig. 8a, b and Supplementary Table 11**). We constructed phylogenetic trees from nuclear and chloroplast genes (**Fig. 4c**, **Extended Data Fig.8c, d**), assigned them to four ancestral populations with ADMIXTURE[31] (**Fig. 4d and h)**, and inferred past population size trajectories by pairwise comparisons of haplotypes with PSMC[32] (**Fig. 4e-g**, **Extended Data Figure 8e**), and searched for shared haplotype blocks with Introblocker[33] (**Fig. 4i**, **Extended Data Fig. 9)**.

PSMC also informs about divergence times: if the ancestral populations to which two haplotypes are assigned to differed in size sometime in the past, those two populations must have split before that point in time[34]. PI365428, a diploid accession from Libya diverged earlier from all other genotypes than these did from each other (**Fig. 4c, Extended Data Fig. 8c-e**). Its population seems to have undergone a recent collapse (**Fig. 4e**), probably owing to the desertification of the Sahara starting around 10,000 years before the present[35] and few incoming migrants across the Mediterranean. Among the sequenced diploids, PI365428 is closest to the autotetraploids (**Extended Data Fig. 8a and f**). It may be a relic of an early diploid population in the eastern Mediterranean before it was replaced by tetraploids. That colonization may have begun as early as 2 Mya (**Extended Data Fig. 8e**). We have to point out that this estimate by PSMC comes with some uncertainty surrounding mutation rates and generation times in a perennial plant. But it is consistent with the divergence 4.5 Mya from barley. FB19-001-1 from the Peloponnese in Greece was an exception. Its lineage separated from Greek diploids so recently that their population size trajectories are largely overlapping (**Fig. 4f**). By contrast, GRA2256-1, a tetraploid from Tajikistan, separated from the Greek diploid lineage around 1.5 Mya (**Fig. 4g**). Furthermore, Greek tetraploids are genetically closer to sympatric diploids than to other tetraploid populations (**Extended Data Fig. 8c, d**) and are also more closely related among each other (**Fig. 4d, Extended Data Fig. 8g and h**). Finally, tetraploid genomes from Greece resemble those of diploids from that country in their TE

profiles (**Extended Fig. 7b**). Taken together, these observations led us to conclude that FB19-001-1 is a member of a tetraploid lineage that arose later and independently from the Asian tetraploids, possibly in Greece. By contrast, GRA2256-1 is an unadmixed representative of an early polyploid lineage. Admixture between both lineages occurs in Greece (**Fig. 4d**), where ancestry from the younger lineage is concentrated in the Peloponnese and western part of the mainland (**Fig. 4h**). Our hypothesis of multiple origins of tetraploid *H. bulbosum* was supported by divergence patterns between haplotypes. The SNP-based distance between older tetraploid lineage peaked at 10 variants per kb corresponding to 1.54 Mya, remarkably close to the split time inferred by PSMC (**Extended Data Fig. 9a**). Ancestral haplotype groups[33] (AHGs) defined by Introblocker were in concordance with ADMIXTURE results. For example, in FB19-028-3, an admixed tetraploid, 19.3 % of the genome was assigned to an AHG prevalent in older tetraploids and 71 % to one common in diploids in the western Mediterranean basin. In contrast, FB19-001-1 had larger contributions from the latter AHG (87.4 %) and only 6.1 % from the tetraploid AHG (**Fig. 4i, Extended Data Fig. 9b**). All this supports the notion that FB19-001-1 is not a chance finding, but is a representative example of interconnectedness of diploid and tetraploid populations. Tetraploidizations in evolutionarily recent times followed by hybridization with older tetraploids may have happened often enough to leave signals that can be picked by population genetic analysis. Present distribution ranges restrict admixture between cytotypes to Greece, where the Pindos mountain range[6] forms the border between di- and tetraploid cytotypes. Future investigations of gene flow across ploidy barriers in bulbous barley will profit from genome sequences of more samples from this potential hybrid zone.

Genomic dissection of resistance-conferring crop-wild introgression

Investigations of tetraploid bulbous barley have implications for plant breeding. Crosses between diploid or tetraploidized barley and tetraploid *H. bulbosum* are the most commonly used method of producing introgression lines[9]. Genome sequences help to confirm the identity of introgression lines and delineate their boundaries. We updated a previous introgression atlas with the positional information obtained from the latest genome assemblies of barley[16] and *H. bulbosum* (**Fig. 5a**, **Supplementary Table 12, and Supplementary Fig. 11 and 12**). We predicted haplotypes of origins for those introgression lines that were derived from A17, one of our pangenome accessions (**Fig. 5b**, **Supplementary Table 12**). The provenance of the donors A17, A40 and A42 has been uncertain. Our GBS data showed that these genotypes are genetically associated with Central Asia populations of the older tetraploid lineage. Long IBD blocks revealed by IntroBlocker show that A40 and A42 share a recent common ancestor (**Extended Data Fig. 9c**). We found that, cumulatively, 1,085 Mb of the *H. bulbosum* genome have been transferred into the barley background at least once, with a cumulative 464 Mb being represented in at least two introgression lines (**Fig. 5a, Supplementary Table 12**). Owing to the fact that both recombination and resistance gene homologs occur preferentially in distal regions, the regions of the *H. bulbosum* genome that were transferred at least twice into barley harbor 65 % of predicted resistance gene homologs of Nucleotide-binding leucine-rich repeat receptors (NLRs) (**Fig. 5c, Extended Data Fig. 10a**). This means that in principle a large fraction of *H. bulbosum* resistance gene diversity can be transferred into barley.

Crop-wild introgressions are often treated as single genetic loci[36]. But recombination between crop and alien chromatin does occur and genetic mapping supports the idea that disease resistances in crop-wild introgressions are attributable to single genes rather than to epistasis which influence ecophysiological traits in tomato-wild introgressions[37]. Introgression lines would come closer to successful deployment if we were able to reduce the extent of alien chromatin and isolate the genes that confer desirable traits. Genome sequences will help. This is illustrated by the *Ryd4^{Hb}* introgression that confers complete dominant resistance to *Barley yellow dwarf virus* (BYDV), an insect-transmitted pathogen that can inflict yield losses of up to 84 % in barley[38]. BYDV also causes disease in other cereal crops, among maize, wheat and oats[39]. In barley, the resistance genes that have been discovered in the primary gene pool of barley[40] provide only partial resistance. The *Ryd4^{Hb}* locus has been delimited to a 65.5 kb interval in the genome of barley cv. Morex that contains two resistance gene homologs of the nucleotide-binding site leucine-rich repeat (NLR) class[41]. A17, the donor of *Ryd4^{Hb}*, is part of our pangenome. To determine which haplotype was introgressed, we assembled the genome of the advanced backcross line JKI-5215, which carries in the background of barley cv. Igri. We found a 32.1 Mb A17-derived segment on the long arm chromosome 3H (**Fig. 5d, Supplementary Fig. 13**). Interestingly, this segment was a mosaic of two haplotypes: the proximal 6.6 Mb match to haplotype 3, while the distal 25.5 Mb match to haplotype 4. Inspection of Hi-C matrices ruled out the presence of a misassembly in A17 (**Supplementary Fig. 14**). A cross-over may have happened in the initial *H. vulgare*-*H. bulbosu*m hybrid progeny, or the actual donor plant was related, but not identical to the sequenced A17. The closest *Ryd4^{Hb}* flanking markers delimited a 692.6 kb interval in JKI-5215 that contained 16 NLR genes, four of which were possibly functional and are therefore considered as candidates **(Supplementary Table 13)**. The sequences of these four genes are between 58.7% and 86.6% identical at the protein level. Two, Ryd4_NLR1 and Ryd4_NLR5 are the closest homologs of and share 80 % of their amino acid sequence with the wheat stem rust resistance gene *Sr35* **(Fig. 5e)**. The *Sr35* locus is also homologous and synteneous to the *Rph13* leaf rust resistance locus of barley[42]. Consistent with diversifying selection often seen at plant resistance gene loci[43], haplotype diversity in the *Ryd4^{Hb}* interval was high in *H. bulbosum*: the JKI-5215 sequence was private to haplotype 4 of A17. Apart from identity-by-descent of three haplotype pairs in A17, GRA2256-1 and PI365428, all haplotypes were singletons. The locus was structurally diverse, mainly owing to duplications of NLR genes. Among the 32 haplotypes of the *H. bulbosum* pangenome, NLR numbers varied from 2 to 23, of which between 2 and 18 had all three typical domains: coiled-coil (CC), nucleotide-binding adaptor shared by APAF-1, certain R gene products and CED-4 (NB-ARC), and leucine-rich repeats (LRR) **(Extended Data Fig. 10c and Supplementary Fig. 14)**. This level of diversity exceeded that previously seen in 76 *H. vulgare* genomes (Jayakodi et al. under review), where NLR numbers at the *Ryd4^{Hb}* locus ranged from 2 to 21; the majority of *H. vulgare* accessions carried less than eight NLRs.

Deployment of *Ryd4^{Hb}* inbarley breeding has been hampered by the presence of a sublethality factor, which has been delimited to a 483 kb region in barley cv. Morex., homologous to a 2.3 Mb interval in the A17 haplotype 4. The recombination event in JKI-5215, which removed lethality, narrowed it further down to 656 kb in A17 and 188 kb in the recurrent parent Igri. Inspection of the gene content is this region

(**Supplementary Table 14**) revealed a homolog of *RecA*, a component of the DNA repair machinery, as a plausible candidate gene. A17 haplotype 4 has three copies, all of which differ in their 5' regions from their barley homolog. In particular, repositioned start codons might affect transcription and translation. These polymorphisms were not found in barley or other *H. bulbosum* haplotypes. The sublethality of *Ryd4^{Hb}* introgressions illustrates that loss of heterozygosity in introgression lines increases the mutation load in crop-wild introgressions lines. A survey of deleterious variants in the *H. bulbosum* pangenome showed that diploid and younger tetraploids contain fewer potentially harmful mutations (**Extended Data Fig. 10d**). Their use as donors might reduce the mutational load of newly generated introgression lines.

Discussion

Pangenomes of crops and their relatives will be assembled in the coming years in increasing numbers, as will "super-pangenomes" – collection of genome sequences for a given crop and all its wild relatives in a wider taxonomic group such as a genus[44]. As sequence reads become longer, haplotype phasing in autotetraploid or heterozygous crop genomes will become easier[45,46]. The contiguity and completeness of resultant assemblies will still depend on the differentiation of haplotypes, which is closely linked to the level of diversity in a species. In the case of *Hordeum bulbosum*, sequence diversity in bulbous barley was large enough to obtain and arrange these into haplotype-resolved pseudomolecules. The availability of chromosome-scale genome sequences for crop wild relatives will be useful for introgression breeding. But genomics cannot overcome all obstacles that stand in the way of a more prominent role of wild relatives in crop improvement. Even allowing for recent progress in engineering plant recombination[47], alien chromatin is unlikely ever to behave like its native counterpart. To find ways around this obstacle, gene mapping might be uncoupled from introgression breeding[48]: thanks to complete genome sequences and fast genome-wide genotyping, geneticists should be able to map traits in wild plants much like they do in crops, either with the help of experimental populations or through association mapping and genome scans in natural populations[49]. Alternatively, existing introgression lines might be used as recurrent parents when making crop-wild crosses. Cross-over in the newly generated hybrids might thus targeted to the loci where alien chromatin is already present in the recurrent parent and thus enrich allelic diversity there. Abundant structural variation at the *Ryd4^{Hb}* and of resistance genes more broadly in the *H. bulbosum* pangenome make this seem a promising avenue to enrich the allelic diversity of barley. Bulbous barley has also been proposed as a target for *de novo* domestication[50]. With genome sequences in hand and enough knowledge about domestication genes in barley[51], it should to edit their homologs in crop wild relatives so as to abolish seed shattering, adapt flowering times to farmers' needs and increase seed set[50,52,53]. Still, the new domesticates might not contribute as much to human sustenance as hoped for[54], but they would be valuable tools for plant scientists and evolutionary geneticists. In any event, genome sequences of crop-wild relatives are foundational resources that underpin each of proposed translational applications.

Declarations

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Contributions

M.M., A.Houben, N.S. and F.R.B. designed research. F.R.B. collected *H. bulbosum* materials. F.R.B. and J.K. grew plant material. A.Himmelbach performed Illumina and PacBio sequencing. J.-W.F. and M.J. assembled genome sequences. J.-W.F. annotated TEs. D.H. contributed GBS data. J.-W.F. and Y.G. analyzed diversity and evolution. H.T. and H.Š. did Bionano genome mapping. T.L., G.H. and M.S. annotated the genome assemblies. H.P., J.-W.F. and N.S. analyzed the $Ryd4^{Hb}$ locus. Y.G. contributed analysis methods. M.C., J.F., Y.-Z.K., S.H. and A.Houben performed and interpreted cytological experiments. J.-W.F., H.P. and M.M. wrote the manuscript with input from all co-authors.

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Figures

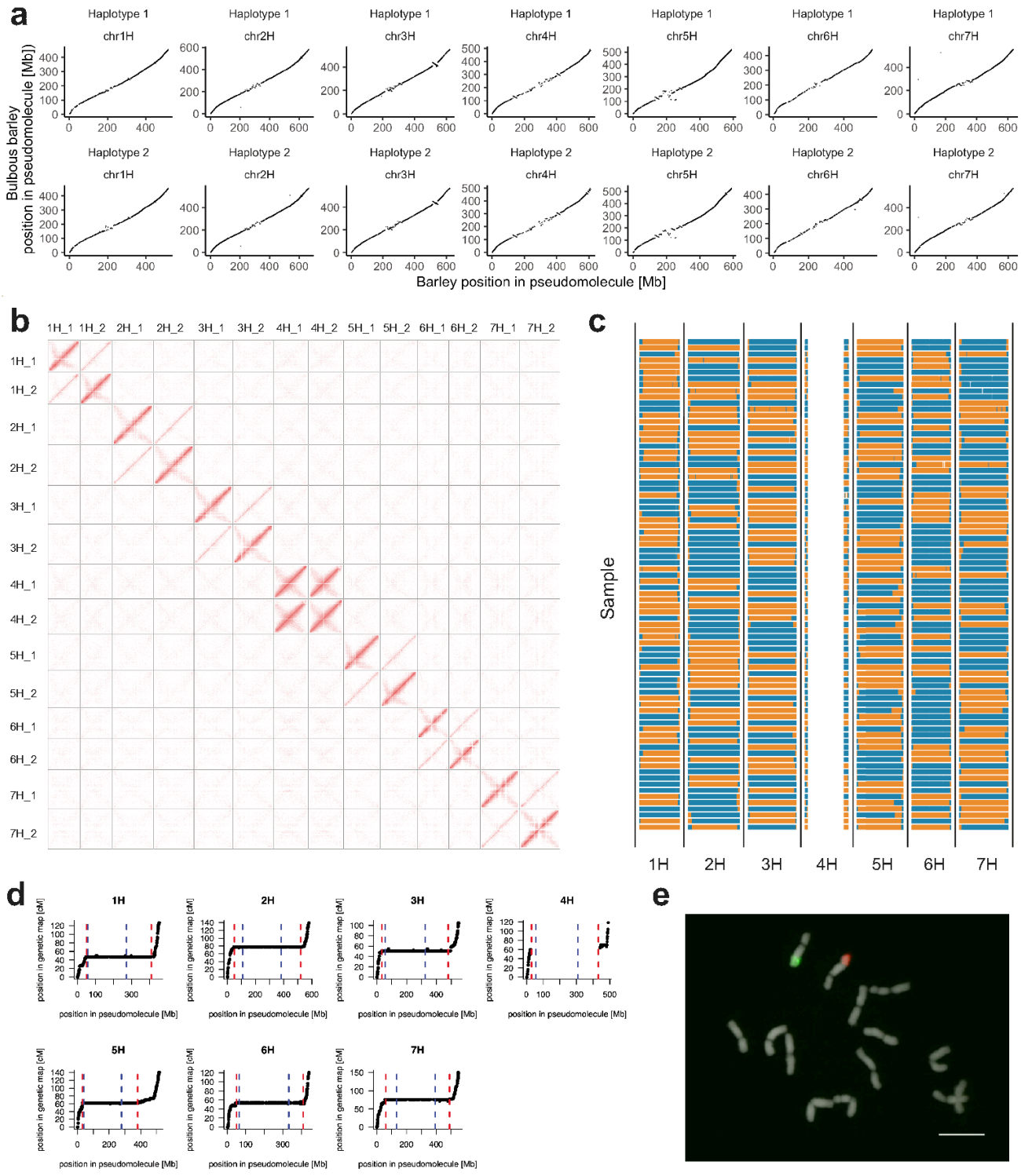

Figure 1

Haplotype-resolved assembly of the diploid H. bulbosum clone FB19-011-3. (a) Chromosome-level sequence alignments between haplotypes 1 (top) and 2 (bottom) of FB19-011-3 and the barley reference cultivar Morex. (b) Diploid Hi- C contact matrix of the FB19-011-3 genome assembly. (c) Graphical genotypes constructed from single-nucleus sequence data of FB19-011-3 pollen. Blue color – haplotype 1. Orange color – haplotype 2. (d) Alignment between the chromosomal pseudomolecules of FB19-011-

3 and the genetic map constructed from the pollen sequencing data shown in panel (c). Dashed red lines indicate the boundaries of the non-recombining regions. The positions of H. bulbosum genes whose barley orthologs are close to the borders of the non-recombining regions in the latter species are marked by blue lines. (e) Haplotype-specific Oligo-FISH in mitotic chromosomes of FB19-011-3. The red and green probes target haplotypes 1 and 2, respectively, of chromosome 5H. Size bar = 10 μm.

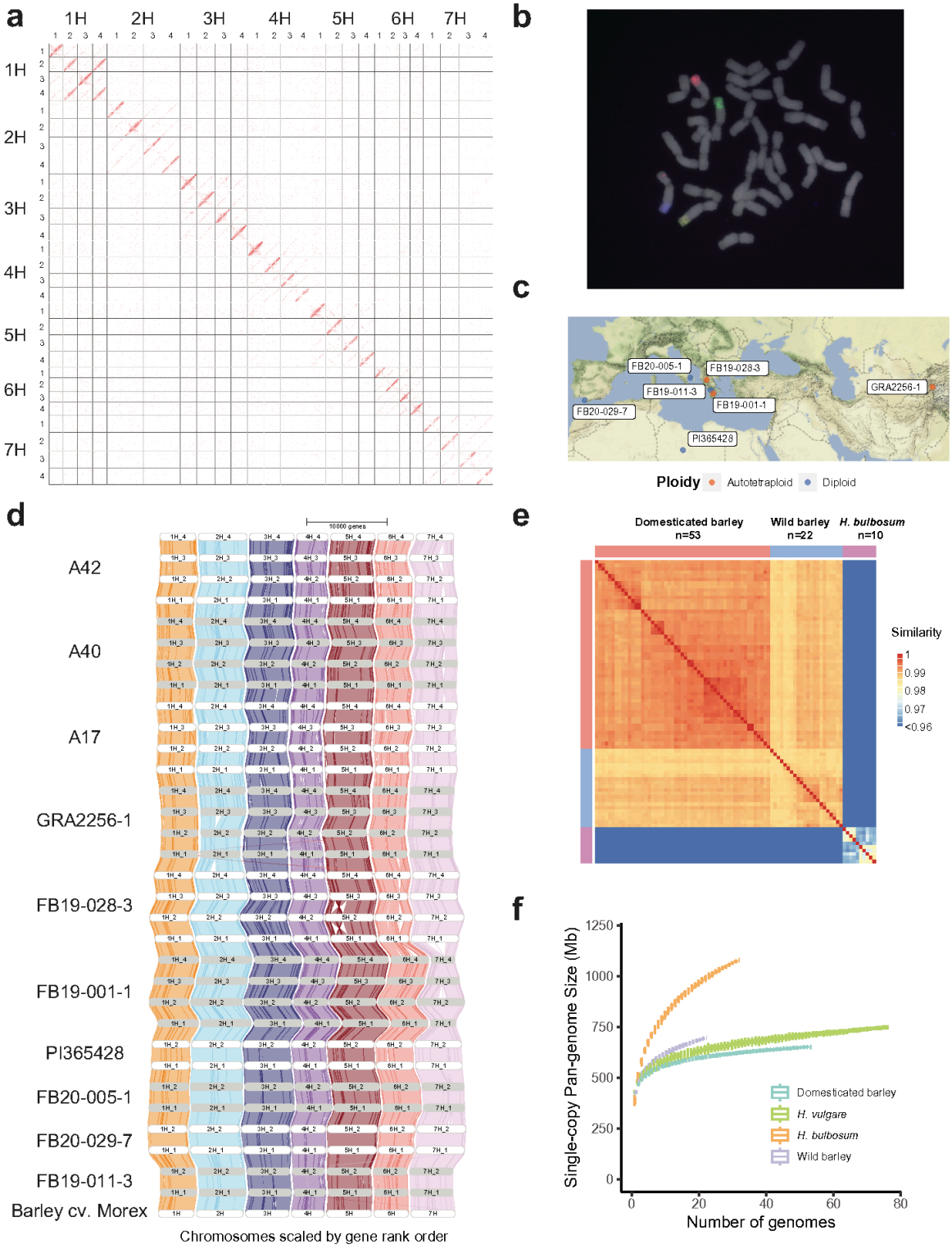

Figure 2

A pangenome of 32 haplotypes from diploid and tetraploid H. bulbosum clones. (a) Hi-C contact matrix of the sequence assembly of the tetraploid clone FB19-028-3. (b) Haplotype-specific Oligo-FISH in mitotic chromosomes of FB19-028-3. The red, green, blue and yellow probes target haplotypes 1, 2, 3 and 4, respectively, of chromosome 5H. Size bar = 10 μm. (c) Map showing the geographic origins of seven H. bulbosum clones that are part of the pangenome. The collection sites of another three accessions are unknown. Colors indicate ploidy. (d) GENESPACE synteny plot between 32 H. bulbosum haplotypes. Grey bars represent chromosomes and are scaled according to the number of genes with syntenic alignments to other genomes. (e) Heatmap showing the similarity of k-mer hashes of H. vulgare and H. bulbosum genome sequences. (f) Pangenome complexity estimated by single-copy k-mers. The curves trace the growth of non-redundant single-copy sequences as sample size increases. Error bars derived from 100 ordered permutations each.

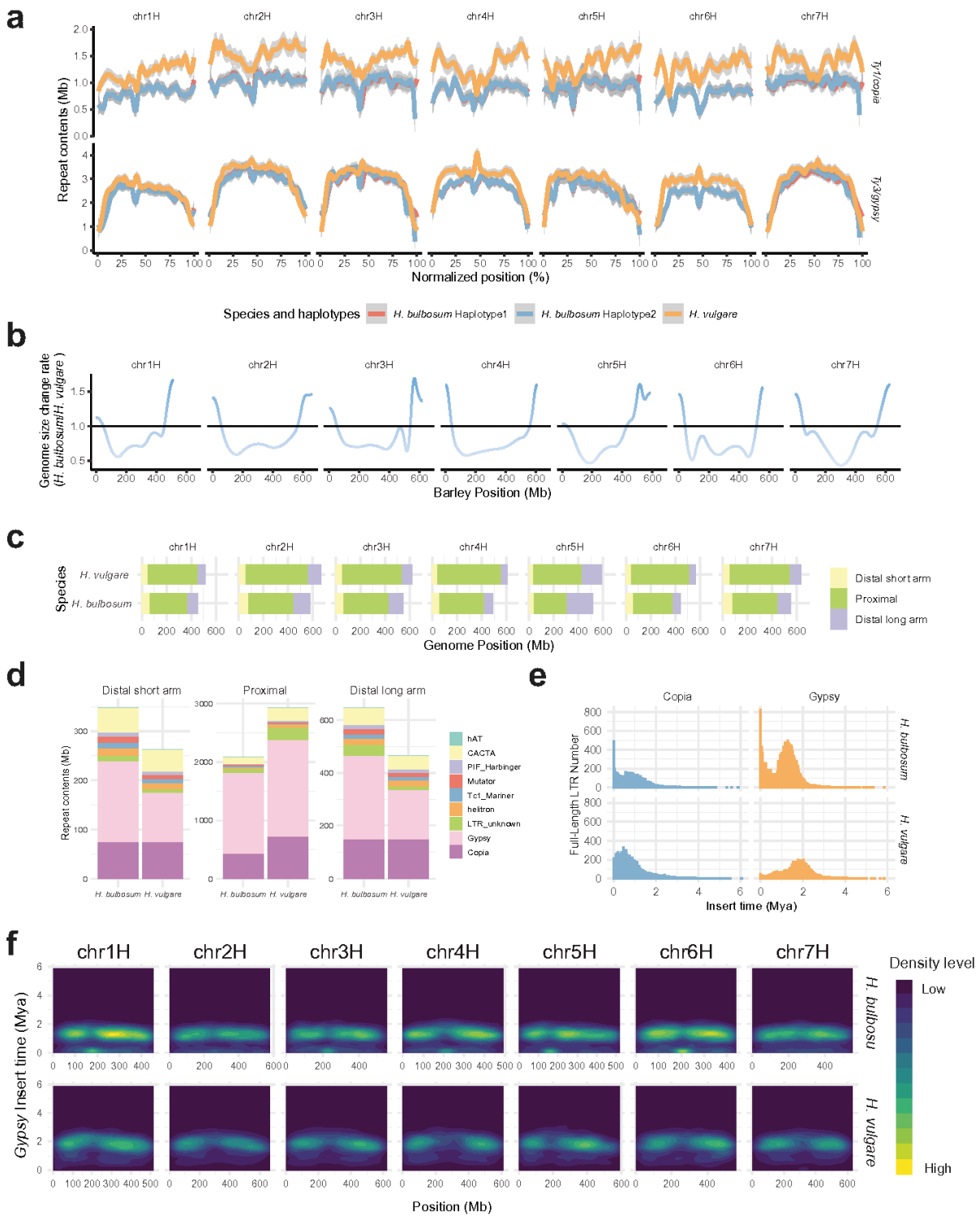

Figure 3

Evolution of transposable elements and genome size in H. bulbosum and H. vulgare . (a) Absolute content of Copia and Gypsy elements along the genomes of both species. Each sequence assembly was divided into 100 bins of equal size. The gray indicates a 95% confidence interval. (b) Local rates of genome size change between H. vulgare and H. bulbosum . (c) Schema showing the partitioning into regions where either the H. bulbosum or the H. vulgare genome is locally expanded. (d) Repeat content

in these partitions. (e) Distribution of insertion times of full-length Copia and Gypsy elements. (f) Twodimensional density plot showing the relationship between insertion time and genomic position of Gypsy elements.

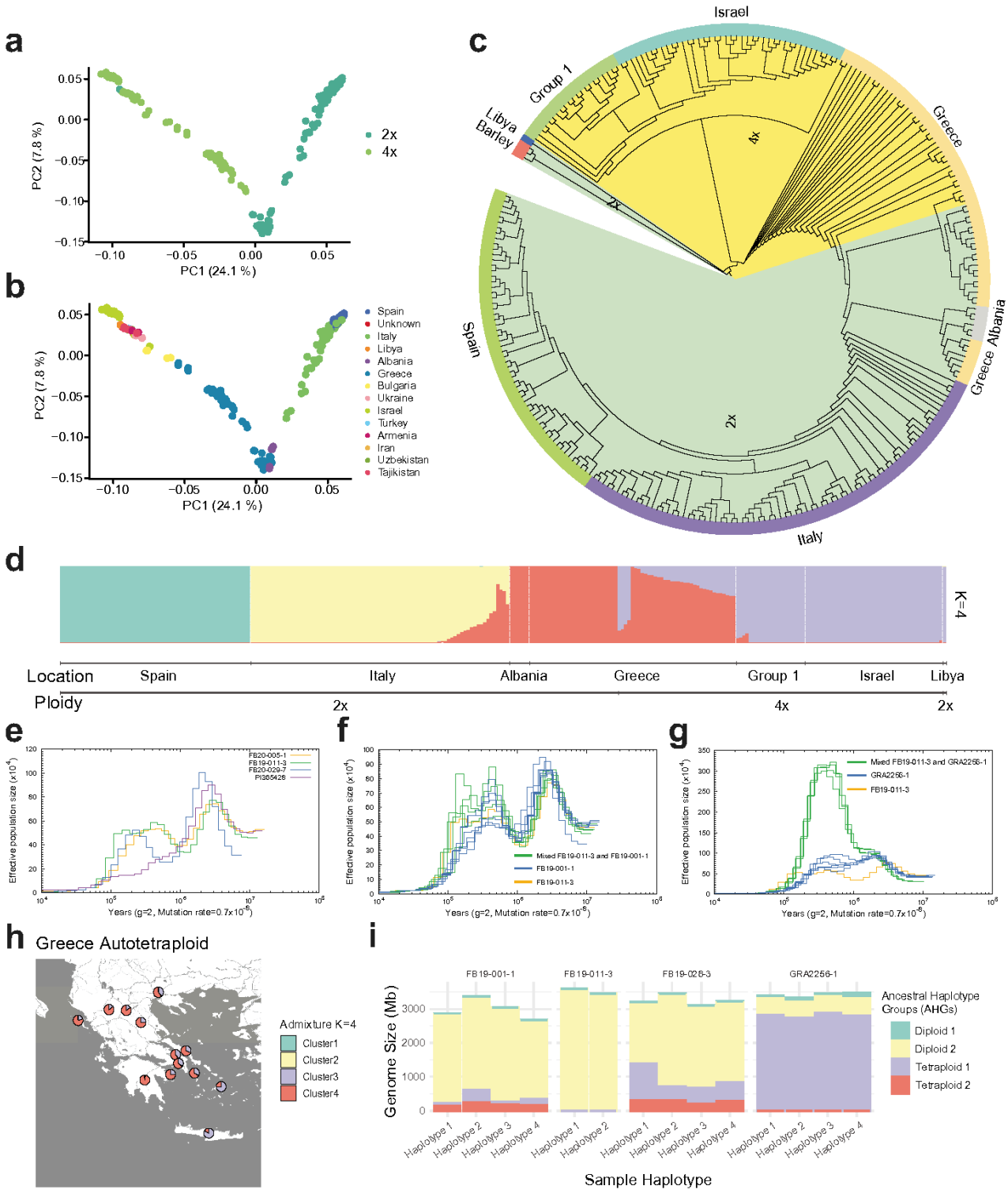

Figure 4

Multiple origins of tetraploid cytotypes. (a, b) Principal component analysis (PCA) of 270 H. bulbosum genotypes colored according to ploidy (a) or country of origin (b). The proportion of variance explained

by the PCs is indicated in the axis labels. (c) Neighbor-joining tree of the same panel. Colors in the outer ring denote geographic origin. Branch color indicate ploidy. Group 1 comprises samples from Armenia, Bulgaria, Tajikistan, Turkey, Ukraine, Uzbekistan, and those of unknown provenance. (d) Model-based ancestry estimation with ADMIXTURE. The barplot shows the ancestry coeffient with k=4 populations. Population structure corresponds to geography and ploidy. (e-g) Population size trajectories as inferred by PSMC from heterozygous genomes. (e) four diploid genomes; (f) the Greek diploid FB19-011-3, the Greek tetraploid FB19-001-1 and synthetic diploids between them; (g) FB19-011-3, the tetraploid GRA2256-1 from Tajikistan and synthetic diploids. (h) Map depicting the collection sites of Greek tetraploids. Pie charts show the ADMIXTURE ancestry coefficients. (i) Ancestral haplotype groups as inferred by Introblocker. The bar plots show the proportion of each Greek H. bulbosum genome that is assigned to one of four ancestral haplotype groups (AHGs).

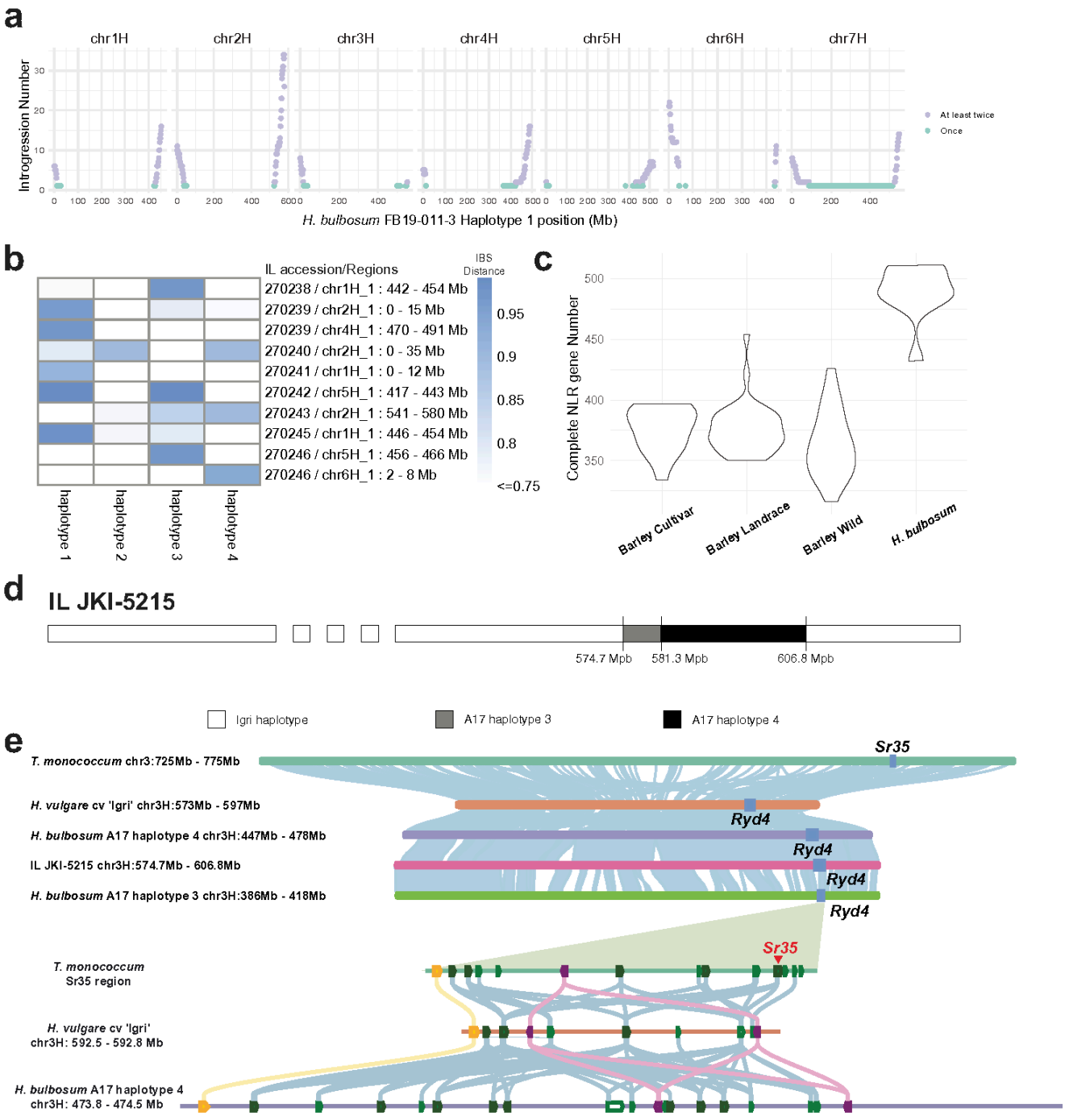

Figure 5

The H. bulbosum pangenome supports introgression breeding in barley. (a) Regions of the H. bulbosum genome introgressed into barley. The y-axis records the number of genotyped barley-H. bulbosum introgression lines carrying any given 1 Mb segment along the H. bulbosum genome. (b) Haplotypes of origin of introgressed segments derived from the clone A17, which is part of the pangenome. The heat map shows the similarity between introgression lines and the four A17 haplotypes in the introgressed segments. (c) Numbers of NLR genes annotated in barley and H. bulbosum haplotypes. (d) Schema

showing the recombined haplotype of the introgression line JKI-5215 in barley background Igri. (e) Microsynteny comparison between the Sr35 interval and the Ryd4Hb interval. The upper panel is the large region comparison among the H. vulgare cultivar ‘Igri’, H. bulbosum A17, T. monococcum TA10622, and introgression line JKI-5215. The blue boxes represent the Sr35 and Ryd4Hb interval. The lower panel is the local synteny between the Sr35 interval and the Ryd4Hb interval in the H. vulgare cultivar ‘Igri’ and in H. bulbosum A17 haplotype 4. The colored horizontal bars represent the length of the intervals. Arrows are annotated genes: yellow coding for S-formylglutathione hydrolase-like proteins, purple for ankyrin repeat-containing protein, green complete NLRs, and white with green outline partial NLRs. Sr35 is identified in red. Syntenic genes are linked when they display at least a fragment with more than 80% nucleotide identity. The color of the links is set to differentiate between gene families.

Supplementary Files

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