Convergent life-history evolution in *Hordeum*: Phylogenomic insights into climatic niche variation and functional genetic differentiation among annual and perennial wild relatives of barley

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Abstract

Annual and perennial life-history strategies have evolved repeatedly across angiosperms, yet the genomic and environmental underpinnings of these transitions remain poorly understood, particularly in grasses. Here, we generated de novo transcriptomes from 82 accessions representing 22 *Hordeum* species, including barley and identified 257 single-copy orthologs present in all accessions to infer a robust phylogeny of the genus. By combining phylogenetic network inference with ABBA-BABA tests, we detected four well-supported cases of interspecific hybridization, three of which coincided with major long-distance dispersal events across continents. Comparative climatic niche analysis indicated that annual *Hordeum* species tend to inhabit environments characterized by higher temperatures and greater interannual temperature variability, as well as increased human disturbance, compared to their perennial relatives, although no consistent differences in precipitation were observed. Using our well-resolved phylogeny as a comparative framework, we analyzed patterns of selection, gene expression, and gene copy-number variation to uncover genomic changes associated with life-history strategy while accounting for phylogenetic non-independence. These analyses yielded 174 candidate genes spanning a wide range of biological functions, suggesting that the genetic architecture underlying life-history evolution is more complex than previously assumed. Candidate genes grouped into six major functional categories, with the most prominent being signal transduction and development, which includes regulators of flowering, dormancy, and meristem activity, metabolic and biosynthetic processes related to carbon allocation and storage, and stress response and defense, reflecting the long-term resilience of perennials compared to the accelerated growth strategies of annuals. Together, our study not only reconstructs the evolutionary history and climatic niche differentiation of *Hordeum* species but also demonstrates that convergent life-history evolution is driven by multifaceted and functionally diverse genetic mechanisms.

Keywords

life-history strategy, perenniality, annuality, comparative phylogenetics, phylotranscriptomics, *Hordeum*, barley, crop wild relatives

Introduction

Plants exhibit remarkable diversity in life history strategies to maximize survival and reproduction in specific environments. A fundamental distinction is between annual and perennial plants. Annuals complete their life cycle within one growing season and are semelparous (monocarpic), reproducing once before dying. Perennials live multiple years, ranging from biennials, with one vegetative year plus one reproductive year, to woody perennials surviving hundreds of years, reproducing annually (Friedman 2020). Although perenniality is often considered the evolutionary default, transitions between annual and perennial life histories occur repeatedly and rapidly across angiosperms. A strong evolutionary signal of the distribution of annual and perennial species in the tree of angiosperms indicate convergent evolution driven by common underlying factors, whether environmental, genetic, or both (Hjertaas et al. 2023).

Understanding the ecological and evolutionary implications of annual versus perennial life history strategies, including how they influence species distributions, community composition, and plant responses to environmental changes, is crucial for predicting ecosystem dynamics and informing conservation and restoration efforts (De Kort et al. 2021). Annuals tend to grow quickly and produce many seeds rapidly, an adaptive bet-hedging strategy for unpredictable or harsh environments (Garnier 1992; Stearns 1992). Many annuals are ruderal, colonizing disturbed habitats rapidly to avoid competition (Grime 1977). Perennials emphasize long-term survival and resource efficiency, investing in structural defenses and resource storage to withstand stresses and persist in stable environments (Chapin et al. 1990; Boege and Marquis 2005). In agriculture, perennials offer benefits such as permanent soil cover reducing erosion (Vallebona et al. 2016) and deeper roots that minimize nutrient leaching (Crews and Rumsey 2017; Lundgren and Des Marais 2020). The advantages of perennial crops are increasingly recognized in sustainable farming (Crews et al. 2018; Zhao and Wang 2024). About two-thirds of global calorie intake comes from annual crops, with five staples (wheat, rice, maize, soybean, barley) dominating (D'Odorico et al. 2014). Barley (*Hordeum vulgare* L.) is a versatile grain, ranking fourth in cultivated area (www.fao.org/faostat). The *Hordeum* genus, in the tribe Triticeae (Poaceae), originated ~10 million years ago in the Eastern Mediterranean and spread into Asia and the Americas (Blattner 2006). Most of ~30 *Hordeum* species radiated in South America (~18 species). Eurasian species relationships are well resolved, but some uncertainties remain regarding the New World phylogeny (Jörgensen 1986; Doebley et al. 1992; Svitashev et al. 1994; Marillia and Scoles 1996; Petersen and Seberg 1998; El-Rabey et al. 2002; Petersen and Seberg 2003; Blattner 2006; Jakob and Blattner 2006; Brassac and Blattner 2015; Jin et al. 2024; Mason-Gamer and White 2024; Feng and Mascher 2025).

Hordeum includes both annual and perennial species, with at least three independent transitions between strategies (Brassac and Blattner 2015). Perennial *Hordeum* species are iteroparous, maintaining above-ground vegetation over winter and initiating growth under long days. A notable exception is Mediterranean perennial *H. bulbosum*, which dies above ground after seed set, surviving as bulbs underground that sprout in autumn rains (Koller and Highkin 1960; Fuerst et al. 2023). This 'pseudo-annual' strategy relies on below-ground organs for clonal propagation (Li et al. 2022). We focus here on the iteroparous perennials maintaining above-ground material. The barley secondary gene pool includes only *H. bulbosum*; other species are tertiary due to crossing barriers (Bothmer et al. 1983; Fedak 1985). Despite such crossing barriers, modern biotechnological methods may enable the utilization of genetic diversity in wild *Hordeum* species, which present a valuable resource for barley improvement and the generation of a perennial barley crop. However, the development of high-yielding, disease-resistant perennial crops remains a significant challenge, requiring ongoing research into the physiological traits and genetic mechanisms that control key differences in annual and perennial growth habits (Chapman et al. 2022).

Studies of trait variation between annual and perennial species span diverse lineages (e.g. De Souza and Da Silva 1987; Herron et al. 2020; Gonzalez-Paleo et al. 2024; Anokye et al. 2025), but genetic insights come mainly from a few models. Brassicaceae is probably the most prominent of these groups, where epi-

genetic control of the genes FLC (annuals) and PEP1 (perennials) dictates seasonal versus perpetual flowering, and modulating their dosages plus two related MADS-box genes can shift plants along an annual–perennial continuum (Bastow et al. 2004; Sung and Amasino 2004; Wang et al. 2009; Amasino 2010; Zhai et al. 2024). Contrary to the intrinsic seasonality that seems to differentiate annual from perennial Brassicaceae, in the tree model hybrid aspen, seasonal growth is regulated by external environmental cues (Böhlenius et al. 2006). Monocot life-history genetics remain elusive: only two perennial crop varieties, PR23 rice (crossed with wild *Oryza longistaminata*) and Kernza wheatgrass, have been developed, with most studies focusing narrowly on rhizome formation (Hu et al. 2003; Hu et al. 2003; Hu et al. 2011; Gruner and Miedaner 2021; S. Zhang et al. 2023). Comparative phylogenetics leverages species or clade phylogenies to test evolutionary hypotheses and, with the explosion of sequence data and advanced methods over the past thirty years, has become a versatile framework for studying phenomena such as trait evolution without prior knowledge of specific genes (Cornwallis and Griffin 2024). However, poorly resolved trees - due to limited genomic data, incomplete lineage sorting, or hybridization - can mislead inferences about trait evolution (Soltis and Soltis 2003). Overcoming these issues requires sophisticated phylogenetic models and high-quality genomic resources. The recent publication of genomes for all 21 diploid and three tetraploid *Hordeum* species (Feng and Mascher 2025) enables comparative genetic studies of the evolution and genetic architecture of various traits, including life-history strategies in the *Hordeum* clade.

In this study, we generated and utilised transcriptomic data from 1-10 accessions of all diploid *Hordeum* species, the 24 recently published *Hordeum* genomes (Feng and Mascher 2025), and publicly available observational data to address three research aims using comparative phylogenomic analyses: (i) increase the resolution of the *Hordeum* phylogeny and investigate ancient inter-species hybridisation events, (ii) uncover the distribution of annual and perennial *Hordeum* species within climatic space, and (iii) explore genetic mechanisms (gene copy number variation, differential expression, divergent signatures of selection) that differentiate between annual and perennial *Hordeum* species.

Results

Hordeum phylogeny and interspecies hybridization

We constructed three phylogenetic trees using distinct datasets and methodologies to assess their topological congruence and investigate various evolutionary phenomena. First, we generated a maximum-likelihood accession-based tree using the combined RNA-seq and reference genome dataset. In this framework, each tip represented an individual sample (accession), allowing us to capture intraspecific variation and evaluate the robustness of species boundaries. This tree was built using 257 single-copy orthologs (scHOGs) shared across all *Hordeum* and outgroup accessions, encompassing a total sequence length of 440,610 bp and 52,026 parsimony-informative sites (Tab. 1). Second, we constructed a Bayesian species tree employing the multi-species coalescent model while simultaneously estimating divergence times. This approach enhances the accuracy of both tree topology and branch length estimation by mitigating biases associated with unequal sampling, intraspecific and gene tree variation, and confounding signals from incomplete lineage sorting and/or hybridization. The species tree was also inferred from the combined RNA-seq and reference genome dataset. However, due to the high computational demands of this approach, the analysis was limited to 90 single-copy orthologs (scHOGs), which had a total alignment length of 225,942 bp and 22,571 parsimony-informative sites (Tab. 1). Third, we constructed a maximum-likelihood tree from the reference genomes of (Feng and Mascher 2025) only (genome accession tree). This tree was mainly a side-product of the analysis of gene CNV and was used for visualization. Note that the data contained only a single accession per species, so while it appears like a species tree, it is an accession-based tree that lacks information about intraspecies variation. The tree

included several distant outgroup species and was based on 380 scHOGs, a total alignment length of 558,769 bp and 115,640 parsimony-informative sites (Tab. 1).

Table 1: Summary statistics of single copy hierarchical orthogroups used for inference of the three phylogenetic trees (for a comprehensive list of statistics see supplementary Tab. S5).

	alignment length [bp]	alignment length without missing data [bp]	variable sites	proportion of variable sites [%]	parsimony informative sites
Accession based tree (257 scHOG; RNA-seq + reference genomes; Fig. 1)	440,610	355,954	86,670	0.2	52,026
Species tree (90 scHOG; RNA-seq + reference genomes; Fig. 2)	225,942	189,900	36,153	0.16	22,571
Genome accession tree (380 scHOG; reference genomes; Fig. 4A)	558,769	453,715	294,500	0,53	115,640

The topographies of the three trees were almost identical (Fig. 1, 2, 4a, S7). Around 10.3 million years ago the *Hordeum* clade split into two large monophyletic groups (95% HPD interval: 8.2, 15.8; for a complete list of exact divergence times, see Fig. S8). One group consisted of the sections Hordeum and Trichostachys with three species growing in Europe, the Mediterranean and central Asia. All species in these two sections are annuals, except for the pseudo-annual *H. bulbosum* (Fig. 1, 2). The other large monophyletic groups included sections Marina and Stenostachys. Section Marina contained only two annual species from Europe and the Mediterranean. Section Stenostachys was the largest section with 16 diploid and two tetraploid species separated into the Asian series Sibirica and the American section Critesion. Only the three youngest of these 18 species (*H. pussilum*, *H. euclaston*, *H. intercedens*) are annual species. Support for interspecies nodes was overall high. In the species tree, only three posterior probabilities fell below 1, with values of 0.99, 0.94, and 0.86 - all within the series Stenostachys. In the accession-based tree, the support values of 88.6/96 UFB/SH-aLRT, the split of *H. comosum* from the other Critesion species was the only interspecies node supported with < 90% bootstrap values (Fig. 1). In the genome accession tree, the node following the split to *H. comosum* had very low support with 34.8/65 (Fig. 4a, S7). This also highlighted the only incongruence of the three trees, which depicted *H. comosum* as the youngest South American species in the species tree (Fig. 2), but as the second youngest lineage, after the clade of *H. muticum* and *H. cordobense,* in the two trees based on individual accessions (Fig 1, 4a, S7). The overall high topographic congruence and node supported of the three trees suggested a solid phylogenetic inference. However, the case of *H. comosum* indicated that evolutionary processes in the closely related Patagonian species complex might be challenging to discern with the utilized data and/or methods.

Interspecies hybridization is one such process that often limits phylogenetic analyses when using strictly bifurcating trees. We expanded our phylogenetic analysis using a two-step approach to investigate reticulations within the *Hordeum* tree. First, we searched for candidate hybridization events by creating ten repetitions of phylogenetic networks with the number of reticulations ranging from 0-10. Eight candidate networks were selected as they showed the best statistical support (Fig. S5, S6). Reticulations that occurred in at least two of the candidate networks were considered potentially true hybridization

events. This analysis yielded five supported interspecies reticulations (R1–R5; Fig. 2). Notably, three of these events (R1–R3) were linked to long-distance dispersal. Specifically, we detected a reticulation between *H. bulbosum* (Eurasia) and the most recent common ancestor (MRCA) of extant American species (R1). Additionally, two reticulations were identified involving *H. californicum* (North America): one with the MRCA of South American species (excluding *H. comosum*; R2) and another with the South American MRCA of *H. muticum* and *H. cordobense* (R3). Surprisingly, we identified only a single reticulation among the youngest, less diverged species (*H. pussilum* → *H. stenostachys*; R5). This event also involved long-distance dispersal, however, it was ultimately refuted in the second step of our procedure, where we calculated D-statistics with ABBA-BABA tests to validate R1-R5. Each candidate reticulation was assessed using multiple ABBA-BABA tests, for which we calculated summary statistics (table inlay Fig. 2; Tab. S6). The single test for R5 was not statistically significant ($p > 0.05$). In contrast, the remaining reticulations were strongly supported, with more than ~90% of their respective tests yielding p-values < 0.05. Variation in substitution rates among species can generate homoplasies that mimic ABBA patterns, potentially leading to false-positive D-statistics.

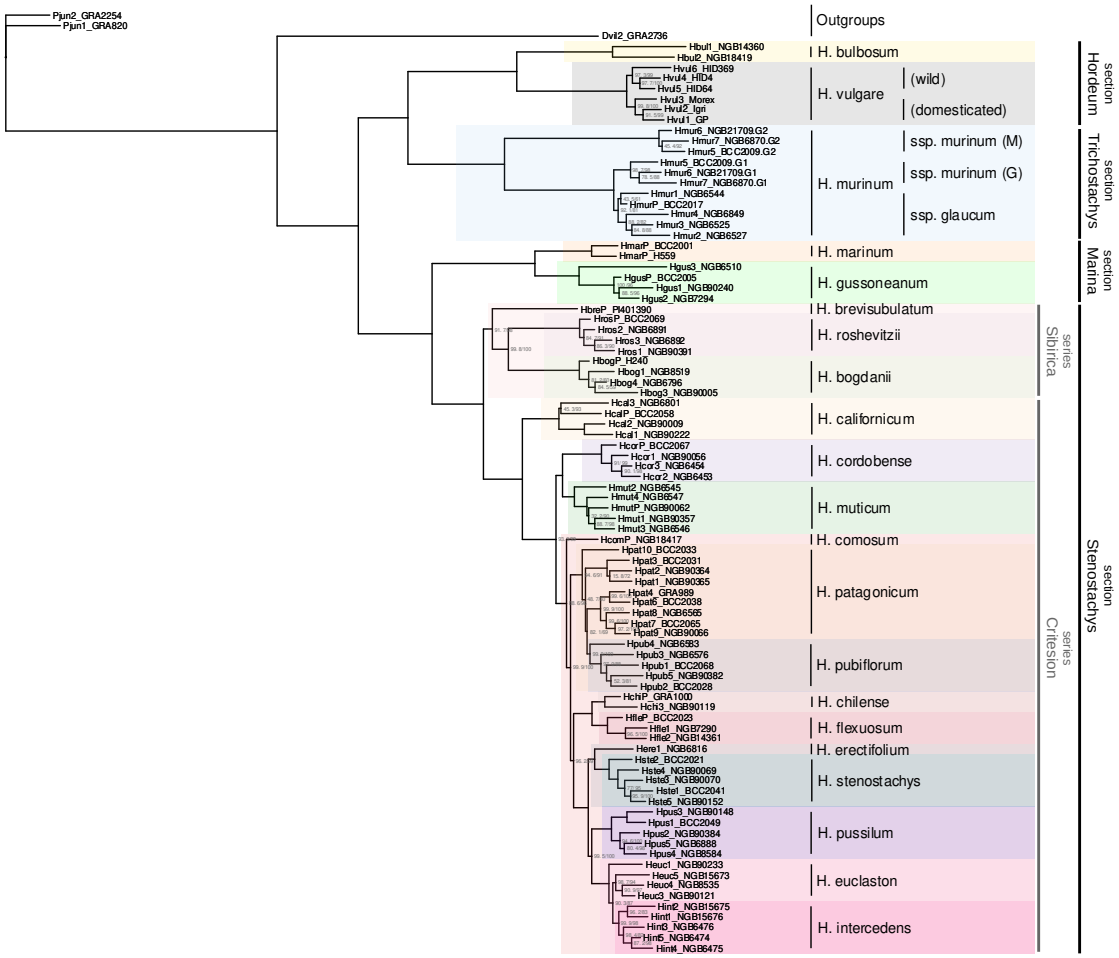
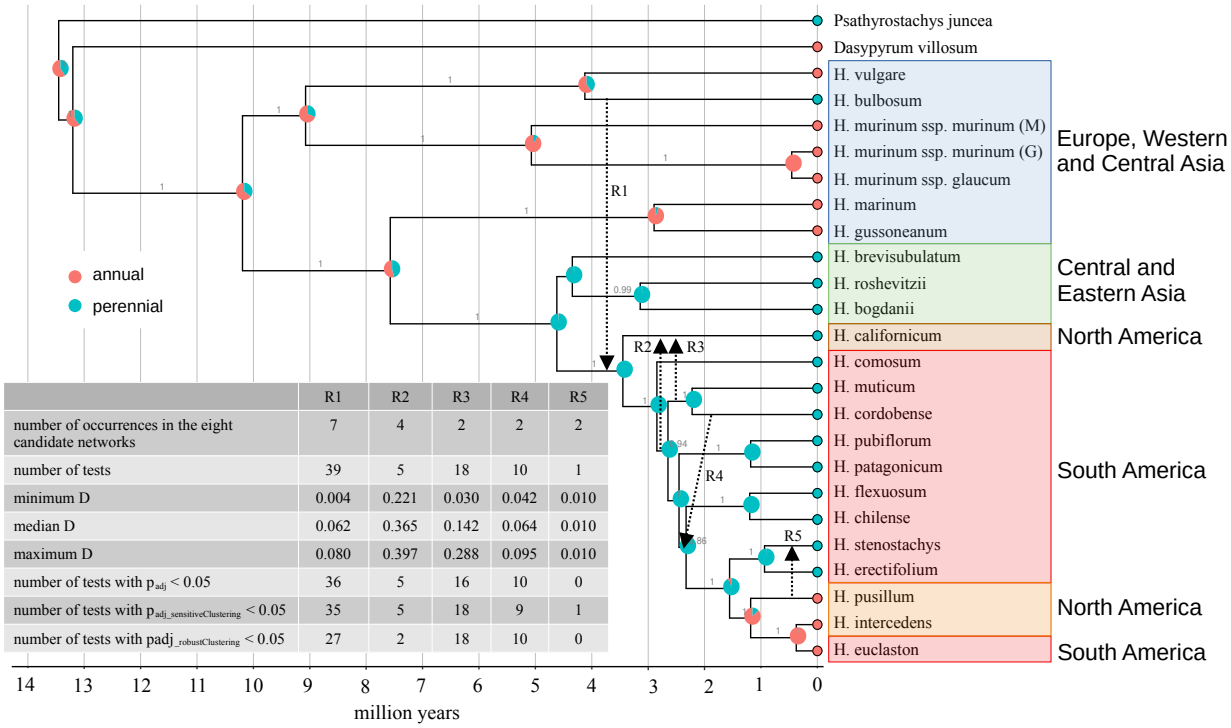


Figure 1: Accession based maximum likelihood tree. Node labels depict UFB/SH-aLRT values. Node labels are only shown if they were not the maximum of 100/100. Infrageneric groupings (sections and series) were defined sensu Blattner (2009).

To address this, we evaluated the degree of clustering of ABBA patterns, distinguishing between those arising from true hybridization events (clustered) and those resulting from homoplasies (randomly distributed). This analysis reinforced the validity of reticulations R1–R4 as genuine hybridization events as the majority of their tests supported a strong clustering tendency of their ABBA patterns (table inlay Fig. 2; Tab. S6). Our analysis initially identified a large number of potential hybridization events (Fig. S6). However, given the statistical challenges associated with these methods, we applied stringent filtering to prior-

itize the sensitivity of our approach. This refinement yielded four well-supported reticulations (Fig. 2). Notably, the majority of these hybridization events -three out of four- were linked to long-distance dispersal, rather than occurring between species growing in proximity. This may indicate an important role of dispersal-driven hybridization in shaping the evolutionary history of *Hordeum*.

Ancestral state reconstruction was performed to estimate the probability of the most recent common ancestor (MRCA) of each clade being either annual or perennial. The analysis indicated that the MRCA of *Hordeum* was most likely annual, though there remained a ~32% probability of a perennial origin (Fig. 2). Transitions between life history strategies appear to have occurred independently three occasions: from annual to perennial along the *H. bulbosum* lineage, again from annual to perennial between ~7.5 and 5.5 million years ago during the expansion into Central Asia, and ~1.5 million years ago along the branch leading to the American annuals (Fig. 2). Notably, similar to the first transition, the second shift was also linked to a dispersal event, this time from South to North America.



	R1	R2	R3	R4	R5
number of occurrences in the eight candidate networks	7	4	2	2	2
number of tests	39	5	18	10	1
minimum D	0.004	0.221	0.030	0.042	0.010
median D	0.062	0.365	0.142	0.064	0.010
maximum D	0.080	0.397	0.288	0.095	0.010
number of tests with $p_{adj} < 0.05$	36	5	16	10	0
number of tests with $p_{adj_sensitiveClustering} < 0.05$	35	5	18	9	1
number of tests with $padj_{_robustClustering} < 0.05$	27	2	18	10	0

Figure 2: Bayesian species tree. Numbers at the nodes are posterior probabilities. Species names are coloured according to their geographic distribution. Tip colours represent life cycle strategy. Pie charts at the nodes represent the probabilities of the ancestral state reconstruction, i.e. the probability that the respective ancestor was annual or perennial. Lines R1-5 represent candidate reticulations. The table summarize the results of the ABBA-BABA test.

Biogeography of annual and perennial *Hordeum* species

Divergent life cycle strategies are widely regarded as evolutionary adaptations shaped by natural selection in response to species-specific environmental pressures, because these strategies influence a suite of functional traits critical to plant fitness, including growth dynamics, reproductive investment, and survival mechanisms. Herein, we investigated macroclimatic variables of *Hordeum* species' publicly available observations to assess whether large-scale patterns of temperature and precipitation are associated with these contrasting life cycle strategies within the clade.

Annual species occupied a substantially smaller space in the bioclimatic PCA than perennials (Figure 3A). The space occupied by perennials covered most of the annuals' and additional areas along the axis of precipitation seasonality (bio15) and temperature annual range (bio7). Accordingly, the climate hyper volumes representing realized niche spaces were larger in perennials (0.93) than in annuals (0.73). Perennials covered 92.3% of the annual niche space and annuals 58.1% of the perennial space. It is

important to note, however, that the analysis included a greater number of perennial (13) than annual (7) species, with the broader climatic niche of perennials largely driven by two specific clades. The first comprised the central and East Asian species *H. bogdanii*, *H. brevisubulatum*, and *H. roshevitzii* (series Sibirica), which inhabit regions characterized by a pronounced continental climate, exhibiting warm summers (bio10), extremely low winter temperatures (bio6), and consequently, an exceptionally large annual temperature range (bio7, 3A). The second group includes *H. comosum*, *H. patagonicum*, and *H. pubiflorum*, native to Patagonia and the Andes, where high precipitation seasonality (bio15) expands the climatic niche of perennials. These two clades consist of closely related species (Fig. 1, 2, 4A), suggesting that the broader niche space observed in perennial species may primarily reflect clade-specific adaptations rather than an intrinsic consequence of their perennial life history strategy.

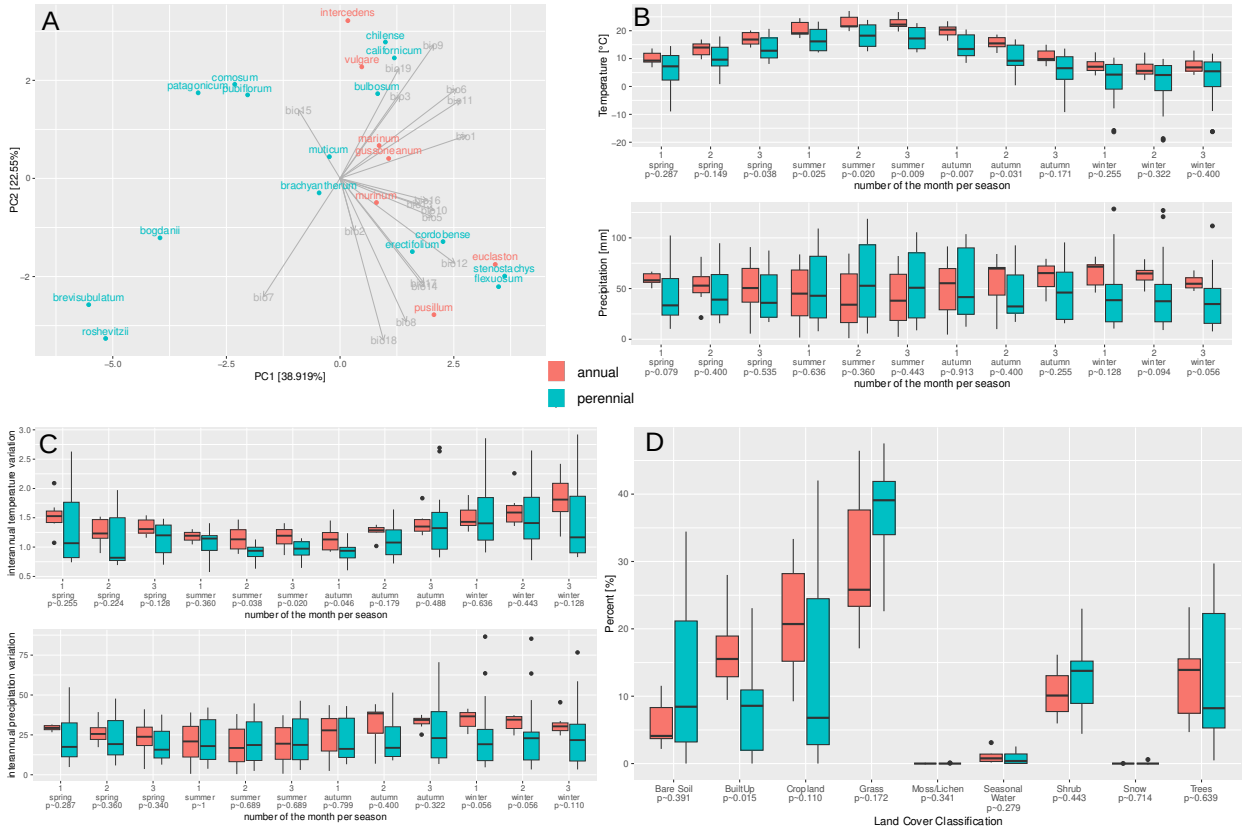


Figure 3: Habitat description of *Hordeum* species. A: Principal component analysis of species by bioclimatic variables. B: Boxplot of monthly mean temperature (top) and precipitation (bottom) of annual and perennial species (excluding *H. bulbosum*) with p-values from Wilcoxon rank-sum tests. C: Boxplot of interannual temperature (top) and precipitation (bottom) variation of annual and perennial species (excluding *H. bulbosum*) with p-values from Wilcoxon rank-sum tests. D: Boxplot of percentage landcover type of annual and perennial species (excluding *H. bulbosum*) with p-values from Wilcoxon rank-sum tests.

Consistent with this perspective, the climatic niche spaces of individual species did not differ significantly between annual and perennial *Hordeum* species ($p \sim 0.36$; Fig. S9). No significant differences were observed in precipitation across any season between the habitats of annual and perennial species, although perennial habitats exhibited slightly lower precipitation levels during winter and early spring. Temperature, on the other hand, tended to be higher in the habitats of annual species throughout the year, yet significant differences ($p < 0.05$) were only detected from late spring to mid-autumn (Fig. 3B). A similar pattern emerged from the analysis of interannual climatic variation. While precipitation did not differ between the habitats of annual and perennial species, annuals appeared to occupy regions with greater interannual temperature variability, particularly from mid-summer to early autumn (Fig. 3C). In terms of land-use associations, annual species exhibited a tendency to occur more frequently near croplands and less frequently in grasslands compared to perennials. However, the only statistically

significant difference in occurrence frequencies was observed for built-up areas. Built-up areas encompass all forms of human-made infrastructure, including residential or industrial buildings, roads, bridges, and other constructed features of the urban or semi-urban landscape. On average, that type of landscape accounted for ~15% and ~8% of land cover in the 100×100 m grid cells surrounding observations of annual and perennial species, respectively (Fig. 3D).

Differences in climatic niche space size between annual and perennial *Hordeum* species were likely shaped by clade-specific adaptations. Meanwhile, climatic distinctions between the habitats of annual and perennial species were not driven by precipitation but were primarily reflected in temperature and its variability from spring to early autumn - the period when annuals flower, set seed, and ripen. Consistent with the reduced temperature predictability of annual habitats, annual species also occupy more human-disturbed environments, which are similarly characterized by low ecological stability.

Divergent genetic determinants in annual and perennial *Hordeum* species

Understanding the genetic determinants that distinguish annual from perennial species provides insights into the functional basis of these life-history strategies, especially when transitions between strategies occur independently multiple times, such as in *Hordeum,* as this helps minimize the confounding effects of ancestral dependencies. We investigated three genetic determinants: divergent selection, differential gene expression, and gene copy number variation, to examine their role in shaping annual and perennial strategies in *Hordeum* species. By integrating these approaches, we aimed to identify genetic variations that are consistently divergent between annual and perennial *Hordeum* species and uncover the genetic architecture (complexity) underlying the different life histories. Together, these will also provide insights into the biological processes and trait complexes accompanying the evolutionary differentiation between annual and perennial *Hordeum* species.

In total, we found 174 candidate genes across the analyses of divergent selection, differential gene expression, and gene copy number variation. We classified 150 genes into five major functional categories, while 24 genes were either coding for proteins with unknown functions or retrotransposon-related proteins (Tab. 2, Fig. 4A). It should be noted that we grouped candidate genes according to conserved domains and their general roles as characterized in *Arabidopsis thaliana* and other model plants, rather than in grasses or, specifically, *Hordeum*. As such, these categories serve only as broad, heuristic classifications to offer a functional overview and should not be construed as precise annotations of individual gene functions. Overall, genes identified across the three analyses were broadly distributed among all functional categories without significant deviations from expected numbers of candidates per category ($p < 0.05$ in chi-squared tests of goodness-of-fit; Fig. 4A). The diversity of putative functions of the identified candidate genes with roles in development, metabolism, cellular organisation, stress response and transcription and translation suggested that a complex genetic architecture underlies the repeated transition between annual and perennial growth in the *Hordeum* clade (Tab. 2). The following sections provide more detailed descriptions of candidate genes and functions in the context of the conducted analyses.

To investigate divergent signatures of selection between annual and perennial *Hordeum* species, we employed two analytical approaches: modelling of the D_n/D_s ratios and the examination of correlations between genes‘ evolutionary rates and the binary trait of life-history strategy. We tested 2908 scHOGs present in at least two accessions per species. Of those 576 and 442 genes were significant in the analysis of D_n/D_s ratios and trait rate correlation, respectively. We took a more conservative approach to determine scHOGs putatively under divergent selection by accepting only genes as candidates that were significant in both analyses, which resulted in 92 candidate scHOGs (Tab. S8). In most of the candidate scHOGs (62), we observed negative trait-rate correlations (Tab. S8), which means that relative evolutionary rates in these genes are, on average, lower in annuals than in perennials. Accordingly, higher D_n/D_s ratios in perennials were observed for these candidate genes. This finding suggests that these scHOGs are under stronger evolutionary constraints in annual than perennial *Hordeum* species. It should be noted that our

290 analysis focused on contemporary signatures of divergent selection, rather than those closely associated
291 with the timing of transitions between life-history strategies. As a result, the direction of these transitions,
292 whether from annual to perennial (~8 - 4.5 myr) or vice versa (~1.5 myr; Fig. 2), should no longer influ-
293 ence the observed signatures. In contrast to the majority of scHOGs (62), nine genes showed positive
294 trait-rate correlations and higher D_n/D_s ratios in annuals. In 20 scHOGs, we observed conflicting signals
295 from the two analyses with negative trait-rate correlations, yet higher D_n/D_s ratios in annuals or vice
296 versa. This was mostly the case when one of the two statistics was significant, but with a very low effect,
297 e.g. RNA.HOG0025477, where the difference between annual and perennial D_n/D_s ratio was significant,
298 yet only very close to 0. These scHOGs may be valid candidates, but their supporting evidence should be
299 considered weaker compared to the other scHOGs.

300 *Table 2*: Summary of the categorization of candidate genes across analyses of divergent genetic mechan-
301 isms; selection, differential expression and gene copy number variation (for a comprehensive list of can-
302 didate genes, their descriptions and categorizations see Tab. S7).

Category	Explanation	Total number of genes per category	Number of genes from selection analysis	Number of genes from differential expression analysis	Number of genes from gCNV analysis
Signal Transduction & Development	Includes receptors, two-component response regulators, and other proteins involved in perceiving signals and controlling developmental programs.	45	25	11	9
Metabolic & Biosynthetic Processes	Contains proteins that participate in the construction and breakdown of cellular components, covering core metabolic pathways and biosynthetic activities.	29	20	4	5
Membrane Transport & Cellular Organization	Groups proteins involved in moving molecules across membranes and those contributing to cellular trafficking and structural organization.	28	16	8	4
Stress Response & Defense Mechanisms	Encompasses proteins that mediate responses to environmental stress, aid in cellular protection, or are implicated in defence responses.	27	12	9	6
Transcription & Translation	Encompasses proteins that together coordinate transcriptional control, RNA maturation and stability, and the assembly and function of the ribosome.	21	13	4	4
Mobile Genetic Elements & Uncharacterised Functions	Groups retrotransposon-related proteins, reverse transcriptase domains, and genes with unknown functions.	24	6	5	13
	Total number of genes per analysis	174	92	41	41

303

304 In addition to expression variation, we analyzed copy number variation to capture dosage effects from
305 both transcriptional and genomic levels, revealing complementary patterns that contribute to the differ-
306 ences between annual and perennial accessions. For this purpose, we searched for gene families where

either all annual species exhibit higher gene counts than perennials, or vice versa. From 29,565 tested gene families, we identified 41 families that satisfied our selection criterion (Tab. S9). Interestingly, in every one of these families, perennials consistently had more copies than annuals. For example, ten gene families had a single copy in annuals, while perennials contained between two and up to nine copies (GENO.HOG0004489, GENO.HOG0005530, GENO.HOG0005718, GENO.HOG0006246, GENO.HOG0007274, GENO.HOG0007793, GENO.HOG0007840, GENO.HOG0008038, GENO.HOG0009567). Additionally, eight gene families were absent in annuals but present in one or more copies in perennials (GENO.HOG0026180, GENO.HOG0026367, GENO.HOG0026903, GENO.HOG0027790, GENO.HOG0029081, GENO.HOG0031918, GENO.HOG0031948, GENO.HOG0031958). Over 50% of these candidates belonged to the ‚Mobile Genetic Elements & Uncharacterized or Unknown Functions' category, within which a majority of genes were from families exhibiting gCNV. This predominance coincides with a high incidence of transposons (e.g. GENO.HOG0002183, GENO.HOG0000833, GENO.HOG0008721) whose capacity for self-replicating makes them especially susceptible to copy-number variation. This category also contained all genes that could not be assigned to other functional classes or for which no molecular function has yet been determined. To further investigate if these copies were also expressed, we examined gene expression for each gene copy within these families. In only six families, we found more gene copies expressed in at least 50% of the perennial accessions than were even present in any annual species. For instance, GENO.HOG0004711, characterized as a senescence regulator, was found in two copies in annuals and in three to four copies in perennials, all of which were expressed (Tab. S8). Another noteworthy gene family (GENO.HOG0007793) contained TEOSINTE BRANCHED1-like genes, which function to integrate hormonal and environmental signals to regulate bud dormancy, suppress branching and flowering, and thus maintain perennial growth cycles (Kebrom et al. 2010; Dong et al. 2019; Zhao and Wang 2024). A limitation of this analysis of expression of paralogs is that we excluded multimapping reads, meaning that gene copies without DNA variation may have been expressed but were filtered out due to our thresholds. In addition, some gene copies may only be expressed under specific conditions, in particular organs, or at certain developmental stages, factors not captured in our experimental setup. As a result, we could only determine the minimum number of gene copies per family that are capable of being expressed. Despite these limitations, our findings demonstrate that gene copy number variation (gCNV) exists between annual and perennial *Hordeum* species. This opens new avenues for more detailed studies, for example, to investigate whether such variation within gene families leads to increased expression, altered dosage, or provides material for evolutionary processes such as neo-functionalization of duplicate genes.

Candidate genes identified through different approaches - analysis of divergent selection, differential gene expression, and gene copy number variation - did not overlap at the gene level. However, these candidate genes shared overlapping functional annotations and belonged to the same biological categories (Table S7). The majority of candidate genes were assigned to the category ‚Signal Transduction & Development' (45/92) and are putatively involved in the control of flowering time, bud dormancy, and meristem maintenance, and thus constitute prime regulators of the divergent life-history strategies observed between annual and perennial grass species. This category contained genes involved in meristem maintenance and organ boundary formation, such as *SCARECROW-LIKE6 (*arath scarecrow protein, RNA.HOG0015866), a Lateral Organ Boundaries domain protein (LOB-domain, GENO.HOG0022843) and NAC domain proteins (GENO.HOG0031918, GENO.HOG0020669, RNA.HOG0010144; (Bolle, 2004; Curaba et al., 2013; Xing et al., 2024)). NAC domain transcription factors also play key roles in regulating leaf senescence and grain development (Christiansen et al., 2016; Christiansen & Gregersen, 2014). Four MYB transcription factors were identified under divergent selection (RNA.HOG0029412), differential expression (GENO.HOG0012470), and genomic copy-number variation (GENO.HOG000448, GENO.HOG0005530). MYB proteins orchestrate transcriptional networks that control cell division, differentiation, and organ patterning in response to both intrinsic and extrinsic signals (Dubos et al., 2010; Ramsay & Glover, 2005).

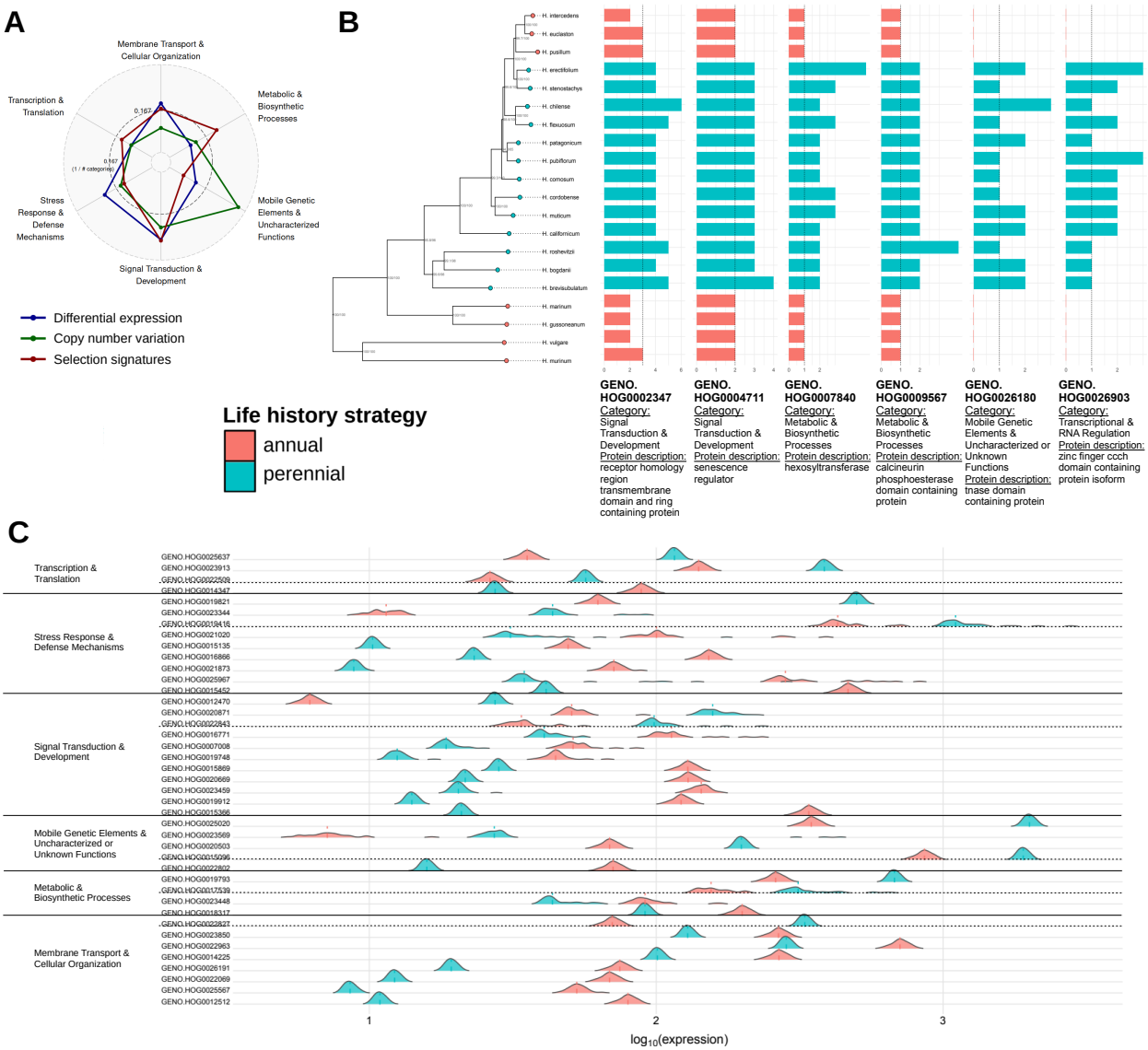


Figure 4: Divergent genetic mechanisms in annual and perennial Hordeum species without *H. bulbosum*. A: Radar plot of the number of genes per analysis assigned to categories. Numbers are relative to the size of the analyses. B: The genome accession tree excluding outgroup species (left). Node labels depict UFB/SH-aLRT values. Node labels are only shown if they were not 100/100. Gene copy number of a selection of candidate gene families (right). For the complete genome accession tree, see Fig. S7. For a comprehensive list of genes that are members of the selected gene families, see Tab. S8 and for the description of the gene families, Tab. S10. C: Ridgeplot of $\log_{10}$ transformed expression of HOGs which are differentially expressed in annual (red) and perennial (cyan) *Hordeum* species. HOGs are sorted according to the categories they were assigned to, and within categories, whether annuals (below the dotted line) or perennials (above the dotted line) showed higher expression.

Noteworthy genes that exhibited divergent selection signatures from the category ‚Transcription & Translation‘ (13/92) were RNA.HOG0026351, RNA.HOG0027556 and RNA.HOG0028859 (Tab. S8), which are ortholog to *Arabidopsis* chromatin remodelling components AtSKIP, AtPIE1 and AtSWP73, respectively. These genes are known to regulate the key floral repressor FLC in *Arabidopsis* (Carter et al., 2018; K. Choi et al., 2007), and their presence among our candidate genes suggests that chromatin remodelers may play a role in shaping life history strategies in *Hordeum* as well.

Another 29 genes of the 92 candidate genes fell into the category ‚Metabolic & Biosynthetic Processes‘, encompassing pathways central to energy production and biosynthesis. For instance, we identified glycosyltransferases (hexosyltransferase: GENO.HOG0007840, alpha glucosyltransferase: RNA.HOG0026057, glycosyltransferase subfamily n-terminal domain containing protein: RNA.HOG0010428), key enzymes that catalyse sugar nucleotide-dependent glycosylation reactions in

carbohydrate metabolism. Several candidate genes are explicitly involved in carbon partitioning between growth and storage in sink tissues, such as beta fructofuranosidase insoluble isoenzymes (invertases: GENO.HOG0002011, GENO.HOG0008038) and a pyrophosphate fructose 6 phosphate 1 phosphotransferase (RNA.HOG0011119; van der Merwe et al. 2010; Duan et al. 2016). Annuals might emphasize metabolic processes that support rapid biomass accumulation and reproduction, whereas perennials may allocate metabolic resources toward maintenance, defence, and storage to ensure survival over multiple seasons. Several genes within the category 'Metabolic & Biosynthetic Processes' - including 4-coumarate CoA ligase, glycerophosphodiester phosphodiesterase, glycosyl hydrolases, glycosyltransferases, pectate lyases, and glucan endo-beta-glucosidases - play direct or indirect roles in cell wall formation and remodelling, thus supporting growth, defense, or stress responses. 'Stress Response & Defense Mechanisms' (27 out of 174 genes) was another large functional category identified and comprised proteins involved in responding to environmental stresses, facilitating cellular protection, and participating in defense-related processes. We identified two scHOGs belonging to the AP2/ERF transcription factor family, which are known to regulate a wide range of abiotic and biotic stress responses, as being significantly upregulated in annuals: GENO.HOG0021020 and GENO.HOG0021873 (Tab. S11; Ma et al. 2024). These genes are orthologous to AtERF105 (AT5G50080) and AtERF53 (AT3G29075), which have been implicated in cold/freezing tolerance and heat/ABA-mediated stress responses, respectively (Hsieh et al., 2013; Illgen et al., 2020). Several genes had functions in oxidative stress response, such as flavin containing monooxigenases (GENO.HOG0016866, GENO.HOG0007274) and Chalcone synthase (GENO.HOG0019416) catalyzing the first step toward production of anthocyanins and other flavonoids, with higher expression in perennials (Dao et al., 2011).

Taken together, our investigation into divergent genetic mechanisms in annual and perennial *Hordeum* species revealed candidate genes across all three genomic features analyzed (signatures of selection, gCNV, differential expression) spanning a wide range of biological functions. This functional diversity suggests that the life-history divergence between annuals and perennials is underpinned by multiple, interconnected genetic processes, rather than a single dominant pathway.

Discussion

Hordeum phylogeny and interspecies hybridization

The phylogenetic relationships among the Old World *Hordeum* species (sect. Hordeum, Trichostachys, Marina, and ser. Sibirica; Fig. 1) have been consistently resolved over decades of research (Jörgensen 1986; Doebley et al. 1992; Svitashev et al. 1994; Marillia and Scoles 1996; Petersen and Seberg 1998; El-Rabey et al. 2002; Petersen and Seberg 2003; Blattner 2006; Jakob and Blattner 2006; Brassac and Blattner 2015; Jin et al. 2024; Mason-Gamer and White 2024; Feng and Mascher 2025). In contrast, phylogenetic relationships within ser. Critesion, particularly among the more recently diversified South American *Hordeum* species, have remained poorly resolved, frequently characterized by polytomies likely resulting from the limited number and type of genetic markers used in earlier studies (Svitashev et al. 1994; Petersen and Seberg 1998; Petersen and Seberg 2003; Blattner 2004; Jakob and Blattner 2006). A notable advance was made by Brassac and Blattner (2015), whose use of 13 genomic loci substantially improved phylogenetic resolution within the genus. While our species tree broadly corroborates their topology, there are some differences, most notably in the placement of *H. pusillum*, which they recovered as sister to *H. stenostachys* and *H. erectifolium*. This topological difference has important implications for reconstructing the evolution of life-history strategies in *Hordeum*, as their scenario would require at least one additional transition between strategies relative to our results. Importantly, node support values in our species tree are markedly higher at all points of topological disagreement with (Brassac & Blattner, 2015) and also exceed those reported by Jin et al. (2024), whose species tree is otherwise fully congruent with ours. This is likely due to the substantially larger data set that we used, with 22,571 parsimony informative sites (Tab. 1) compared to 2,852 and 7,064 in the data set of Brassac & Blattner (2015) and Jin et al. (2024), respectively. Given the larger dataset and consistently higher node support in our analyses, we

consider our phylogenetic reconstruction to represent a more robust and reliable representation of species relationships in *Hordeum* compared to previous studies. As such, it provides a more suitable framework for inferring the evolution of life-history strategies within the genus. More broadly, these results underscore the critical importance of accurate phylogenetic relationships in comparative phylogenomic analyses, as incorrect assumptions about species' evolutionary histories can generate misleading signals, obscure true patterns of trait evolution, and ultimately lead to erroneous inferences about the genetic architecture underlying phenotypic diversity.

As increasingly large genomic datasets and more sophisticated analytical approaches are applied to phylogenetic inference, our understanding of species relationships continues to improve. However, it has also become evident that while additional data can strengthen support for many relationships, certain phylogenetic conflicts persist despite these advances (Mason-Gamer and White 2024). We observed a similar pattern in *Hordeum*: although the placement of most species was consistent across all our reconstructed trees, the position of *H. comosum* remained unstable, varying among alternative topologies. This phylogenetic instability is also reflected in other recent studies, where *H. comosum* was either recovered as sister to *H. patagonicum* and *H. pubiflorum*, forming the youngest independent South American lineage, as in our species tree, or as the second youngest independent South American lineage - following the divergence of the *H. muticum* and *H. cordobense* clade - as observed in both of our accession-based trees (Brassac and Blattner 2015; Jin et al. 2024; Feng and Mascher 2025). Since *H. comosum* is nested within the large clade of perennials, this instability is not relevant for our comparative phylogenomic analyses of life-history strategies. However, it may present challenges for studies focusing on other traits where the early South American lineages play a more important role.

The causes of this phylogenetic uncertainty remain speculative. A limited number of accessions per species - as used for *H. comosum* herein, in Jin et al. (2024) and Feng and Mascher (2025) - may constrain the ability accurately determine species relationships. A limited number of genetic markers - as used by Brassac and Blattner (2015) - can increase the risk of stochastic error and reduce the power to resolve complex evolutionary relationships. Additionally, it is well recognized that evolutionary histories are not always adequately represented by strictly bifurcating tree models (e.g. Bapteste et al., 2013; DeSalle & Riley, 2020; Morrison, 2005). We identified a large number of candidate inter-species introgressions in *Hordeum* using a phylogenetic network approach (Fig. S5, S6). However, the robust detection of introgression remains inherently challenging, as signals of gene flow are often subtle, restricted to specific genomic regions, and can be confounded by incomplete lineage sorting (Hibbins and Hahn 2022). To mitigate these challenges and minimize false positives, we focused on introgression events that were consistently recovered across multiple networks and further validated them using D-statistics. This conservative approach yielded four statistically well-supported cases of inter-species introgression. Notably, three of these events coincided with long-distance dispersal events - from Europe/West Asia to East Asia/North America (R1) and from South to North America (R2, R3; Fig. 2). None of our detected introgressions involved *H. comosum*, and therefore, reticulations fail to account for the inconsistent placement of this species in both our and earlier reported trees. Previous studies investigating inter-species introgression in *Hordeum* have identified a variety of putative gene flow events; however, none of the introgression events reported, including those detected in our study, have been independently corroborated (Jin et al. 2024; Feng and Mascher 2025). This apparent discrepancy is most likely attributable to the fact that different studies targeted distinct genomic regions, applied varying statistical frameworks, or differed in sampling strategies. This observation highlights a fundamental challenge in studying introgression in large-genome taxa like *Hordeum* (~3–5 Gbp in diploid genomes; Feng and Mascher 2025) and underscores the necessity for continued and integrative research efforts. A single study alone is unlikely to capture the full complexity of introgression dynamics in a genus of this size and evolutionary history. Despite the methodological challenges and limited congruence among studies, the accumulating evidence suggests that inter-species hybridization may have occurred repeatedly and across broad geographic and

phylogenetic scales throughout the evolutionary history of *Hordeum*. The combination of previous reports on putative introgression events, our results herein, and the presence of numerous polyploid *Hordeum* species - many of which originated from hybridization between distantly related lineages across the genus (see results herein: Fig. S7; Brassac and Blattner 2015; Jin et al. 2024; Feng and Mascher 2025) - collectively support a scenario where inter-species hybridization may have been a recurrent and pervasive evolutionary process in *Hordeum*.

Biogeography and evolution of annual and perennial *Hordeum* species

While annuality in plants is often considered to be the result of adaptations to unpredictable environments, high temperatures or seasonal droughts (Stearns 1992), empirical evidence shows that not all plant genera consistently exhibit clear associations between life-history strategies and such climatic characteristics of their native habitats (Boyko et al. 2023; Poppenwimer et al. 2023). Annual and perennial *Hordeum* species did not exhibit any significant differences in precipitation patterns, regardless of the time of year, in our analysis - a pattern generally consistent with findings for *Hordeum*'s subfamily Pooideae (Boyko et al. 2023), but not with those reported for the broader Poaceae family (Poppenwimer et al. 2023). Summer and early autumn temperatures, on the other hand, were significantly higher in habitats of annual Hordeum species (Fig. 3B, C), suggesting that high summer temperatures favoured annual *Hordeum*. Accelerated development and early maturation are common escape strategies of grasses facing terminal heat stress (Loka et al. 2019; Jagadish 2020; Bahrami et al. 2021). An association between the maximum temperature of the warmest month and annuality was the only consistent correlation with life history strategy observed across flowering plant taxa in (Boyko et al. 2023), suggesting a broadly generalizable pattern linking thermal extremes to the prevalence of annuality in plants - similar to the pattern observed in *Hordeum* reported herein.

We did not observe significant differences in niche space between annual and perennial *Hordeum* species when analyzed individually (Supplementary Fig. S9). However, when annuals or perennials were considered jointly, perennial species occupied a substantially larger niche space (Fig. 3A). This pattern was primarily driven by the series Sibirica, which expanded the niche breadth of perennials due to their association with environments characterized by a high annual temperature range (bio7) and low minimum winter temperatures (bio6). Notably, the correlation between low winter temperatures and perenniality has been identified as a general pattern not across all flowering plants, but within the Pooideae subfamily (Boyko et al., 2023). According to life-history theory, perennial species possess an adaptive advantage in environments where adult mortality is low but seedling mortality is high (Cole 1954; Charnov and Schaffer 1973). This theoretical framework may help explain the observed transition from annuality to perenniality during *Hordeum*‘s expansion from Western Asia - characterized by a Mediterranean climate - into Siberia’s continental climate (Fig. 3). In this context, harsh Siberian winters may have acted as an environmental filter, selecting against annuality in the winter type *Hordeum* species of Europe and South-West Asia due to elevated seedling mortality. Conversely, regions where annual *Hordeum* species are most prevalent, such as Mediterranean climates, are characterized by high summer temperatures that compromise adult plant survival, while mild winters facilitate seedling establishment and survival, thereby favouring annuality.

Low levels of ecological predictability have been linked to the prevalence of annual species, as their rapid life cycles may enable them to complete reproduction before the onset of unfavourable conditions (Stearns 1992; Poppenwimer et al. 2023). In our study, annual *Hordeum* species were found to occur more frequently in areas near human-made infrastructure (built-up areas; Fig. 3D). Although anthropogenic disturbance has not directly driven transitions between life history strategies in *Hordeum*, such disturbances are typically more intense near human settlements and may exert selective pressure favouring an annual growth habit. Furthermore, we observed greater interannual variability in summer temperatures in regions inhabited by annual *Hordeum* species (Fig. 3C). Together, these findings suggest that annuality in

Hordeum may confer an adaptive advantage under conditions of environmental unpredictability, consistent with life-history theory and previous findings in other plant taxa.

Divergent genetic mechanisms in annual and perennial *Hordeum* species

We used three complementary genome-wide approaches, scans for divergent selection, differential gene expression, and gene copy-number variation, to identify orthologous genes that consistently distinguish annual from perennial *Hordeum* species. By integrating phylogenetic relationships from our species tree and accounting for at least three independent life-history transitions within *Hordeum*, we controlled for shared ancestry and pinpointed genetic signatures genuinely linked to life-history divergence. This led to 174 high-confidence candidate genes across diverse functional categories, suggesting a convergent genetic basis for annual and perennial strategies in *Hordeum*. Genetic convergence mirrors known phenotypic patterns: across angiosperms, annuals typically exhibit faster growth, earlier flowering, and higher reproductive allocation, whereas perennials favor conservative resource use (Garnier 1992; Lundgren and Des Marais 2020; Hjertaas et al. 2023). Similar trends, such as earlier flowering and greater early biomass in annual *Hordeum*, have been observed, though leaf-economic traits are less consistent (Anokye et al. 2025). Thus, our discovery of convergent genetic changes parallels broader ecological syndromes. Most previous genetic studies of annual–perennial divergence lacked phylogenetic replication, making it hard to separate true convergence from shared ancestry. Exceptions in *Arabis* and *Draba* used independent annual-perennial sister lineages to uncover convergent mechanisms (Heidel et al. 2016; Kiefer et al. 2017). For instance, Heidel et al. (2016) found five gene families retained in Arabis and Draba perennials but lost in annuals. Notably, our *Hordeum* gCNV analysis recovered families with matching predicted functions for three of these: oxidoreductases (GENO.HOG0003613, GENO.HOG0005718, GENO.HOG0007274), an F-box protein family (GENO.HOG0002661), and zinc-finger proteins (GENO.HOG0001510, GENO.HOG0009714, GENO.HOG0026903; Tab. S9). Despite such examples, phylogenetically replicated studies of genetic convergence of life-history strategies remain scarce, and to the best of our knowledge, none have hitherto been reported in grasses. In this context, our work contributes to a growing but still small literature that applies replicated, phylogenetically informed comparisons to pinpoint genetic changes specifically linked to life-history transitions, rather than unrelated lineage-specific evolution.

The repeated emergence of annuals and perennials in *Hordeum* supports the idea that life-history transitions involve relatively simple genetic changes. This view originates from theoretical arguments suggesting that traits evolving repeatedly over short evolutionary timescales are unlikely to involve complex genetic architectures. The view was further reinforced by QTL studies that often find only a few major-effect loci (Friedman and Willis 2013; Gruner and Miedaner 2021) and by work in Brassicaceae linking annual-perennial shifts to altered expression of only a few MADS-box genes (Melzer et al. 2008; Li et al. 2024; Zhai et al. 2024). Contrasting this view, our candidate genes in *Hordeum* span a broad array of functions. While studies in the Brassicaceae suggest that simple genetic changes can drive transitions along life-history strategies (Zhai et al. 2024), the situation in *Hordeum* appears more complex. Either the genetic basis of perennialism is more intricate in *Hordeum*, or, more likely, successful, stable transitions in nature require coordinated changes across multiple genes and pathways that together produce the full suite of associated traits. In the context of the increased interest in perennialization of annual crops (Crews et al. 2018; Zhao and Wang 2024), this has significant implications: breeding perennial crops that are agronomically competitive to their annual counterparts may demand broad, system-level genetic modifications, not just tweaks to a few key genes.

Signal-transduction and developmental regulators formed the largest functional category among our candidates. We detected a *Hordeum* SCARECROW-LIKE6 (SCL6) ortholog under divergent selection. In *Arabidopsis*, SCL6 overexpression delays flowering and extends lifespan, sustaining meristem activity (Xing et al. 2024). A TEOSINTE BRANCHED1 (TB1)-like gene family was also under divergent selection. TB1 functions as an integrator of hormonal and environmental signals, regulating bud dormancy,

suppressing branching and flowering, and thereby contributing to the maintenance of perennial growth cycles (Dong et al., 2019; Kebrom et al., 2010; Zhao & Wang, 2024). Transcription factors in the NAC and MYB families, key mediators of ABA- and JA-dependent stress, dormancy, and senescence pathways, also exhibited divergent signatures of selection. For instance, *Arabidopsis* ORE1 (a NAC) is a master regulator of senescence (Chun et al., 2023), and barley NAC promoters often contain ABA- or GA-responsive elements (Arif et al., 2025), suggesting shifts in the timing of senescence and dormancy between strategies. We further identified three chromatin-remodelling genes under divergent selection, orthologs of Arabidopsis SKIP, PIE1, and SWP73, which participate in SWR1 and SWI/SNF complexes that regulate the floral repressor FLC in *Arabidopsis* (Aslam et al., 2019; Carter et al., 2018; Choi et al., 2017; Crevillén & Dean, 2011). Mutations in these genes in Arabidopsis reduce FLC expression and cause early flowering (Bastow et al., 2004; Hepworth & Dean, 2015), suggesting epigenetic regulation of flowering may also involved in life-history divergence in *Hordeum*. Regulation of senescence is also closely linked to source-sink dynamics. As discussed above, transcription factors like NAC and MYB influence aging processes. Early activation of these genes in annuals may promote senescence to remobilize nutrients into developing seeds, while perennials may delay their expression to prolong leaf function. Beyond transcriptional control, we also identified several metabolic enzymes under divergent selection. Notably, multiple glucosyltransferases showed such signatures and several studies highlight the role of glucosyltransferases in modulating source-sink dynamics (Stein & Granot, 2019). Similarly, UDP-glycosyltransferases (UGTs) fine-tune source–sink balance by regulating hormone levels and sugar availability (Ross et al., 2001). Another enzyme that showed divergent signatures of selection was pyrophosphate–fructose-6-phosphate 1-phosphotransferase (PFP). PFP has been shown to play a critical role in regulating carbon partitioning between growth and storage in sink tissues in annual (rice; (Duan et al., 2016) and perennial (sugarcane; van der Merwe et al., 2010) grasses. Our findings suggest that a key component of the genetic differences between annual and perennial *Hordeum* species lies in carbon metabolism and source-sink allocation. Annuals tend to prioritize rapid growth and seed development by channelling photosynthates quickly, while perennials allocate more resources toward maintenance, defense, and long-term storage. Perennial species are expected to exhibit higher stress resilience since they need to maintain their vegetative material over several years while annuals for example avoid late season stress by completing their life cycle early (Calone et al., 2022; Chapman et al., 2022; Lundgren & Des Marais, 2020). Consequentially, we also found several candidate genes categories as ‚Stress Response & Defense Mechanisms‘. For example, we observed differential expression of two genes from the AP2/ERF transcription factor family, which are known to regulate a wide range of abiotic and biotic stress responses (Ma et al., 2024). Conversely, higher expression in perennials were observed for Chalcone synthase (CHS). CHS is a key enzyme in flavonoid biosynthesis, catalyzing the first step toward production of anthocyanins and other flavonoids, and it is strongly induced by stress (Dao et al., 2011). Divergent signatures of selection were observed for a chloroplastic ClpB chaperone, which play crucial roles in cell adaptation to high-temperature conditions (Mishra & Grover, 2016). Our candidate genes in these functional categories align with classic annual - perennial life-history trade-offs. ‚Signal Transduction & Development‘ genes set the developmental program by controlling the timing of flowering, dormancy, and meristem activity. Genes related to „Metabolic & Biosynthetic Processes“ execute resource-use strategies by modulating carbon flux, storage compound synthesis, and metabolic priorities. In addition, the enrichment of ‘Stress Response & Defense Mechanism’ genes supports the notion that perennials invest more in long-term tissue protection and stress resilience, enabling them to survive across multiple growing seasons. In contrast, annuals often evade late-season stress by accelerating their life cycle, prioritizing rapid growth and reproductive output.

612 **Material and Methods**

613 **Plant Material and RNA sequencing**

614 We compiled a collection of 82 genebank accessions, which included 19 *Hordeum* and two outgroup
615 species (Supplementary Tab. S1). The seeds of these accessions were sown in freshly prepared soil
616 mixture, 99.6 % (v/v) Mini tray soil (MIM800, Balster Einheitserdewerk, Fröndenberg, Germany) and 0.4
617 % (v/v) Osmocote exact standard 3-4M (Scotts Company LLC). Seeds were stratified in the dark for
618 14 days at 4 C° and subsequently germinated under long day conditions in walk-in phytochambers (day:
619 16h, 22 °C; night: 8h, 18 °C). Seedlings that developed 2-3 true leaves were moved to vernalization for 9-
620 14 weeks (day: 8h, 4°C; night: 16h, 4°C). After vernalization, seedlings were moved back into walk-in
621 phytochambers (day: 16h, 22 °C; night: 8h, 18 °C) and grown to maturity.

622 Tissue samples were taken from roots, leaves, stems and inflorescence. Root samples were taken during
623 re-potting of plants into 7x7cm pots around 14 days after transfer out of vernalization. Leaf samples were
624 taken from the second or third fully developed true leaf at Zeitgeber (ZT) 13.5-15.5 and 21.5-23.5.
625 Inflorescence samples were taken in the booting stage (Zadok stage 60-69) sampling only the central part
626 of the inflorescence (middle third of the inflorescence). Stem samples were taken at the same time from
627 the same tiller as the inflorescence sample. We removed the leaves from the stem and sampled one
628 random internode Note that not all plants flowered within one year, and therefore, we could not sample all
629 tissues of all plants (Tab. S1).

630 RNA was extracted from all tissue samples using a RNeasy kit (Qiagen, Hilden, Germany). The extracted
631 RNA of roots and leaves were pooled, and the RNA of stem and inflorescence were pooled for each
632 accession with equal amounts of RNA per tissue sample. Both pools per accessions were sent to
633 Biomarker Technologies (BMK) GmbH (Münster, Germany) for strand-specific poly-A enriched mRNA
634 library preparation and sequencing of min. 6Gb/sample on an Illumina Novaseq 6000 with 150bp paired-
635 end reads.

636 **Transcriptome assemblies**

637 Raw reads from the leaves-root and the stem-inflorescence pools were merged into a single fastq file per
638 accession. After assessing the raw reads' quality using FastQC (v0.11.9; Andrews, 2010) and MultiQC
639 (v1.7; Ewels et al., 2016), the raw reads were filtered with fastp (v0.23.2; Chen et al., 2018; Fig. S1)
640 using the following parameter settings: -l 50, --detect_adapter_for_pe, --cut_right, --
641 cut_right_mean_quality 20, -c.

642 Transcripts were assembled *denovo* using rnaSPADES (v3.9.1; Bushmanova et al., 2019; Prjibelski et al.,
643 2020) with default settings. Additionally, a genome-guided assembly was done with Trinity (v2.2.0;
644 Grabherr et al., 2011). We ran Trinity three times with kmer 20, 26 and 32 and the parameters --
645 SS_lib_type RF and --genome_guided_max_intron 2500. The bam files required for genome-guided
646 assembly were created by mapping the filtered reads against the reference genomes of the respective
647 species (Tab. S1) except for the outgroup *Psathyrostachys juncea* (Fisch.), which due to the lack of a
648 reference genome, was mapped against the reference of the second outgroup *Dasypyrum villosum* (L.
649 Borbás; X. Zhang et al. 2023). Our collection contained two tetraploid species, *Hordeum bulbosum* (L.;
650 autotetraploid) and *Hordeum murinum* ssp. *murinum* (L.; allotetraploid). For autotetraploid *H. bulbosum*
651 we arbitrarily chose genome 1 from the reference genome and treated the two accessions as diploids.
652 Reads from *H. m.* ssp. *murinum* accessions were mapped against the allotetraploid genome. Reads that
653 mapped to genomes 1 and 2 (herein denoted as 'G' and 'M', respectively, *sensu* Rajhathy & Morrison,
654 1962) were extracted and separately mapped against the separated G or M genome reference. The
655 separated reads from the G and M genome of each *H. m.* ssp. *murinum* accessions were treated like two
656 diploid samples for further procedure. The mapping procedure was done using STAR (v2.7.10a; Dobin et

al., 2013) with default settings. In a subsequent filtering step with SAMtools (v1.3.1; Danecek et al., 2021), only uniquely mapped reads were retained.

The four assemblies per accession were merged into a single fasta file and mapped against the same reference genome used for read mapping using Minimap2 with the '-ax splice' option (v2.24; H. Li, 2018). The longest transcript was extracted from the resulting bam file with AGAT (v0.8.0; Dainat, 2020). We used BLAST+ (v2.2.26; Camacho et al., 2009) with the parameters -max_target_seqs 1, -outfmt 6 and -evalue 1e-5 to create a database based on the concatenated proteins of the barley variety Morex (v3; Mascher et al., 2021) and *D. villosum*. This database was input for Transdecoder (v5.5.0; Haas, 2024) which we used with default parameters to predict open reading frames and collections of the longest transcript, coding and protein sequences.

The sequence production and transcriptome assemblies' workflow was summarized in Fig. S2.

Detection and processing of orthologs

The predicted protein sequences from the 85 assemblies (77 accessions from diploid species + 2 *H. bulbosum* + 3 *H. murinum* G-genomes + 3 *H. murinum* M-genomes) were complemented with protein sequences from 13 accessions from the utilized *Hordeum* reference genomes (Feng and Mascher 2025). Thus, the final collection consisted of 95 accessions plus 3 'artificial' diploid *H. m.* ssp. *murinum* samples from in total 21 *Hordeum* and two outgroup species (Tab. S1, S2). The protein sequences of this collection were used as input for OrthoFinder (v2.5.4; Emms & Kelly, 2019) with parameters -M msa and -T fasttree. The resulting gene families will have the prefix "RNA." in the following to distinguish them from the second OrthoFinder analysis described below.

After inspecting the resulting tree created by OrthoFinder, seven accessions did not cluster at their expected positions in the tree (marked red in Fig. S3). Note, that these seven accessions could not represent intra-species variation as they clustered far away from the approximate position of their species in the tree. For example, *H. chilense* is a South American species, yet two of its samples clustered deep in the clade of an Asian species (*H. roshevitzii*). We consider it to be likely, that all of these seven plants did not belong to the taxa they were assigned to and, therefore, represented faulty phylogenetic signals.. Since these accessions may have biased the detection of orthologs, we removed them and repeated the analysis with the pruned set of 87 accessions plus three 'artificial' diploid *H. m.* ssp. *murinum* (Tab. S1) and continued with this pruned set of accessions.

After running OrthoFinder again with the pruned set of accessions, we extracted single-copy hierarchical orthogroups (scHOGs) which were present in all accessions and aligned their coding sequences to create a multiple sequence alignment (MSA) for each scHOG. Alignment was done with MAFFT (v7.407; Katoh et al., 2002). Each MSA was trimmed using Clipkit (v1.4.1; Steenwyk et al., 2020) with default parameters to increase the signal-to-noise ratio and improve the MSAs' quality for subsequent phylogenetic analyses.

Phylogenetic tree inference

We created partition schemes from scHOGs using PartitionFinder (v2.1.1; Lanfear et al., 2017) using raxml, a greedy search across all models and aicc as a decision criterion for model selection.

Fasta files were created that contained the scHOG sequences from the determined best fitting partitioning scheme and partition-trees of each partition were created using IQtree (v2.2.0; Minh et al., 2020) with 1000 ultrafast bootstrap values (UFB). Substitution models were determined with ModelFinder (Kalyaanamoorthy et al. 2017). The partition-trees and the MSAs were used to estimate a number of properties and sorting the partitions according to their usefulness for phylogenetic inferences following the method of genesortR (Koch 2021).

None of the partitions exhibited any properties that suggested they may introduce a bias (Fig. S4). Hence, all partitions were concatenated and used to create an accession-based phylogenetic tree using the maximum-likelihood approach implemented in IQtree (v2.2.0; Minh et al., 2020) with the best fitting models of nucleotide substitution determined by ModelFinder (Kalyaanamoorthy et al. 2017). We used 100,000 UFB and 100,000 bootstrap replicates of the SH-like approximate likelihood ratio test (SH-aLRT; Guindon et al., 2010) to test the robustness of the inference. Additional applied parameters were -m MFP+ and --sampling GENESITE.

A Bayesian analysis with parallel inference of the species tree and divergence times is computationally expensive and was not feasible with all scHOGs. Therefore, we only used coding sequences of the first 50 partitions (= 90 scHOGs), according to the order determined by the genesortR analysis, for Bayesian tree inference with the StarBeast3 model (v1.1.8; Douglas et al., 2022) of BEAST (v2.7.5; Bouckaert et al., 2019). For the analysis with BEAST, we implemented the substitution models determined to best fit to the 50 partitions by PartitionFinder in the above described analysis. As clock model of the species tree, we used a relaxed clock and estimated its standard deviation and clock rate. As a model of speciation, the calibrated Yule model was used. Estimations of mutation rates in wild *Arabidopsis thaliana* (L.) populations were reported to range between 2 and 5 $x10^{-9}$ $site^{-1}year^{-1}$ (Exposito-Alonso et al. 2018). Even though it is likely that the mutation rates in perennial *Hordeum* species are slightly lower, these estimates are a sufficient proxy for a prior distribution. Therefore, we defined the priors for the clock rates of the species tree and the partitions as a log-normal distribution with a mean (μ) of 0.0035 and a standard deviation of 0.9 (note, these values were transformed to $site^{-1}$ [million years]$^{-1}$). We created three MRCA priors for time calibration. A normal distribution with μ=19.4 and =6 at the split of *P. juncea* and *D. villosum* (Bernhardt et al. 2017), a normal distribution with μ=12 and =3 at the split of *D. villosum* and *Hordeum* (Brassac and Blattner 2015; Bernhardt et al. 2017) and a normal distribution with μ=9.23 and =1.3 at the *Hordeum* root (Bernhardt et al., 2017; Brassac & Blattner, 2015) . Chain lengths were 700 million, storing every 4,000 samples. We used CoupledMCMC (1.2.0; Müller & Bouckaert, 2020) with four chains. All other parameters were left at default. The corresponding xml file can be found in the supplementary material. We ran this analysis three times with different seeds and inspected the log files in Tracer (v1.7.2; Rambaut et al., 2018) to ensure convergence. The three independent runs were combined with Logcombiner (v2.7.6; Rambaut et al., 2018) discarding 10% of the trees of each run as burn-in to create the final collection of tree samples. Treeannotator (v2.7.6; Drummond & Rambaut, 2007) was used to summarize tree samples to a maximum clade credibility tree with 0% burn-in and 0.5 posterior probability limit.

Ancestral state reconstruction was done to estimate the probability that an ancestor was either annual or perennial. Species' life-history strategies were defined based on (Bothmer et al. 1995) (Tab. S1) after their description was verified by common garden experiments in the Botanical Garden Düsseldorf (Anokye et al. 2025); Longitude: 51.188324, Latitude: 6.800582). We defined annuals as species that senescent within one year after germination while perennial plants survived more than one cycle of seasons and transitioned from vegetative to reproductive growth at least twice. The R package 'ape' was used for this analysis (Paradis and Schliep 2019).

Interspecies hybridization

We extracted all coding sequences from scHOGs in Hordeum which were present in at least two accessions per species or in one accession for the species which were represented by only a single accession. This resulted in a set of 2,909 scHOGs, which in the following will be referred to as the 'two-accession set'. The sequences were aligned and trimmed using the same approach outlined above with the scHOGs present in all accessions.

Phylogenetic network inference was conducted with the maximum pseudo-likelihood approach developed for SNAQ (Solís-Lemus et al. 2017) and implemented in the Julia (v1.10.2; Bezanson et al., 2017)

package 'PhyloNetworks' (v0.16.3; Solís-Lemus et al., 2017). Genetrees from the aligned coding sequences of the two-accessions set were estimated with IQtree (v2.2.0; Minh et al., 2020) using the same procedure as outlined above for creating the input of genesortR (Koch 2021). Species quartet concordance factors were calculated from these genetrees. We ran SNAQ 10 independent times with different seeds, allowed reticulations ranging from 0-10 and the species tree as a guide tree. We considered all networks with a pseudo log-likelihood below a certain threshold as candidate networks. This threshold was arbitrary. Yet, in all models, there was a steep increase in pseudo-log-likelihood between networks below and above this threshold, suggesting a clear decrease in statistical support for networks above the threshold (Fig. S5). Reticulations that appeared in at least two networks were considered candidate reticulations (Fig. S6).

For SNP calling, we used the reference genome from the species that was on average the least diverged from all other species. The most suitable reference genome was determined by calculating patristic distances between all accessions from the accessions tree. The species with the lowest average patristic distances to all other species was *H. comosum* (J.Presl), which therefore was used as the common reference genome. Filtered reads from all accessions subjected to RNA sequencing were mapped against the *H. comosum* reference genome (Feng and Mascher 2025) using STAR (v2.7.10a; Dobin et al., 2013) with default settings. In subsequent filtering steps with SAMtools (v1.3.1; Danecek et al., 2021), only uniquely mapped reads were retained. Variant calling was done with BCFtools (v1.10.2; Danecek et al., 2021) and default parameters. We kept only biallelic SNPs without missing data, QUAL ≥ 30, GQ ≥30, DP ≥ 5 and for heterozygous SNP calls DP ≥ 30. VCFtools (v0.1.16; Danecek et al., 2011) and BCFtools (v1.10.2; Danecek et al., 2021) were used for variant filtering. The 13 included accessions from Feng and Mascher (2025) were included by extracting the gene space defined in the gff files of the corresponding species from the respective reference genomes. These sequences were mapped against the *H. comosum* reference genome using Minimap2 with the asm20 option to account for the divergence between species (Li 2018). Only sequences with MAPQ ≥ 30 were used to call biallelic, homozygous SNPs with QUAL ≥ 30. The two VCF files were merged and the merged file was used for the subsequent procedure.

Linkage disequilibrium (LD) pruning was done with PLINK (v 1.90b7; Purcell et al., 2007). Windows of 50 kb were shifted by five variants, and SNPs with $r^2 \geq 0.5$ within one window were removed. The pruned SNP set was used as input for Dsuite (v0.5r53; Malinsky et al., 2021) to conduct ABBA-BABA tests for candidate reticulations found with SNAQ (Solís-Lemus et al. 2017). In all tests, *Dasypyrum villosum* was the outgroup species. In Dsuite, we used the --ABBAclustering parameter to test whether ABBA patterns in tests with significant D tend to cluster together (Koppetsch et al. 2024).

Biogeography of annual and perennial *Hordeum* species

We downloaded publicly available georeferenced observations of the diploid *Hordeum* species included in our phylogeny from GBIF (GBIF.org 2022), the New York Botanical Garden (NANSH; http://sweetgum.nybg.org/science/ih/; accessed 16.08.2022) and the North American Network of Small Herbaria (https://nansh.org/portal/collections/index.php; accessed 16.08.2022). Since public databases of plant occurrences are often contaminated with faulty entries, we restricted data points of each *Hordeum* species to their geographic distribution as previously described (Bothmer et al. 1995). Additionally, we selected one random observation from observations that occurred within a 10km radius to avoid over representation of more frequently monitored areas. Climate variables were downloaded for all remaining observations from Worldclim 2 (Fick and Hijmans 2017) with a resolution of 2.5 minutes. Landcover descriptions were retrieved from the European Union's Copernicus Land Monitoring Service (Land Cover 2019, version 3, 100m raster resolution; Buchhorn et al., 2020). This cleaned data set was used for the subsequent procedure (Tab. S3).

Due to the large variation of data points per species, we calculated the mean of each environmental variable and continued with the species means to avoid biases due to over- or under-representation of specific species. Median absolute deviations were calculated as an estimator for interannual temperature and precipitation variation.

Climate niche spaces were approximated by the hypervolume of the climate variables calculated using dynamic range boxes with geometric means of individual variables implemented in the R package 'dynRB' (v0.18; Junker et al. 2016). This procedure was done for all accessions across annual species and for all accessions across perennial species. Additionally, hypervolumes were calculated from accessions of each species separately, except for *H. erectifolium*, where only a single accession is available.

Wilcoxon rank-sum tests were used to test the significance of each climate niche and variable between annuals and perennials. The entire procedure was done in R (v4.3.3; R CoreTeam, 2021).

As mentioned above, we focused on iteroparous species that continuously maintain above-ground vegetative material and, therefore, excluded the pseudo-annual *H. bulbosum* from this analysis. However, to be comprehensive, we also conducted the analysis a second time with *H. bulbosum* included. Yet, these results are not discussed and are only presented in the supplementary files. The same procedures were followed for the analyses of gene copy number variation, differential expression, and selection explained below.

Gene copy number variation in the context of life-history strategies

For analyses of gene family sizes, we only used protein sequences from all reference genomes published by Feng and Mascher (2025) and added six outgroup species: *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, *Sorghum bicolor*, *Brachypodium distachyon* and *Dasypyrum villosum* (Tab. S4). The protein sequences of this collection were used as input for OrthoFinder (v2.5.4; Emms & Kelly, 2019) and processed the same way as outlined above for the protein sequences from the transcriptome assemblies and the selected accessions from Feng and Mascher (2025). The resulting gene families will have the prefix "GENO." in the following to distinguish them from the first OrthoFinder analysis described above.

Single copy HOGs were extracted from the output and used for the same phylogenetic analyses like described above for the protein sequences from the transcriptome assemblies and the selected accessions from Feng and Mascher (2025) except for the species tree inference, i.e. alignment and trimming → creation of partition → estimating gene properties for phylogenetic inference → accession based phylogenetic inference. The resulting accession tree will be called 'genome accession tree' hereafter.

The outgroups were removed from the assignment of proteins to gene family (N0.tsv) done by OrthoFinder (v2.5.4; Emms & Kelly, 2019). We then counted the number of genes per gene family for all diploid Hordeum species and filtered this data set. The data set contained seven annual *Hordeum* species, and we wanted to reduce the computational expenses by excluding as many gene families with large variations of presence/absence within annuals or perennials because these were unlikely to be functionally linked to differences between life history strategies. We allowed 1-species error margin and, hence, retained only gene families where at least 6 species had a gene count of ≥ 1.

A gene family was considered a candidate if the gene count of the smallest family in an annual species exceeded the gene count of the largest family in a perennial species or vice versa. The procedure was done in R (v4.3.3; R Core Team, 2021) using base functions. The entire procedure was done twice, once with all accessions and once without *H. bulbosum* accessions.

835 **Differential gene expression in the context of life-history strategies**

836 For the analysis of differential gene expression, only diploid *Hordeum* accessions subjected to RNA
837 sequencing were used. Read counts were derived from the mapped reads using HTSeq (v2.0; Putri et al.,
838 2022). Gene counts of accessions were sorted according to assignments to scHOGs done by OrthoFinder
839 (v2.5.4; Emms & Kelly, 2019) for the analysis of gene copy number variation described above. Genes'
840 tagwise dispersion and size factors (using "TMM") were estimated with the R package 'edgeR' (v4.2.2;
841 Chen et al. 2025). Significant differential expression between annual and perennial accessions were tested
842 by fitting a generalized mixed linear model with negative binomial errors and a log link function
843 (ngGLMM) which was implemented the R package 'glmmSeq' (v.0.5.5; Lewis et al., 2024). The
844 phylogenetic relationship among accessions was included as random effect variables to account non-
845 independence of accessions. The phylogenetic relationship was determined by the first three principal
846 coordinates of the cophenetic distances of the ML based accession tree from the 87 pruned set of
847 accessions while excluding the 13 accessions from Feng and Mascher (2025), because they were not
848 subjected to RNA sequencing. P-values were corrected for multiple testing with the Benjamini-Hochberg
849 method (Benjamini and Hochberg 1995). Genes were considered differentially expressed (DE) candidates
850 if their corrected p-value was ≤ 0.01 and their $|\log_2(\text{fold change})|$ was ≥ 1.

851 Gene features including GO-terms of these final candidate genes were extracted from the *Hordeum*
852 *comosum* accession (NGB18417) because it was the only accession that had a sequence in all scHOGs of
853 the two-accession set and available gene features from Feng and Mascher (2025).

854 GO-term enrichment analysis was done for biological processes using a Fischer's exact test with all genes
855 used for the analysis as background and candidate genes as genes of interest. The analysis was done in
856 the R package 'topGO' (v2.56.0; Rahnenfuhrer & Alexa, 2024). GO-terms were considered significant if
857 the p-values were ≤ 0.05. The entire procedure was done twice, once with all accessions and once without
858 *H. bulbosum* accessions.

859 **Selection signatures in the context of life-history strategies**

860 Coding sequences from the two-accession set were subjected to codon-aligned sequence alignment using
861 MACSE (v2.07; Ranwez et al., 2018) with parameter -local_realign_init 1 and -local_realign_dec 1. Each
862 of these sequences were used as input to CODEML implemented in PAML (v4.10.7; Yang, 2007) to
863 model D_n/D_s across the accession tree, including only accessions from *Hordeum* species, which allowed
864 us to control for phylogenetic non-independence. We ran the null model (M_0) and the branch model (M_{alt}),
865 where accessions from annual species build the foreground branches.

866 The two-accession set was used to create gene trees with the 'estimatePhangornTreeAll' function with
867 GTR as model of nucleotide substitution implemented in the R package 'RERconverge' (v0.3.0;
868 Kowalczyk et al., 2019). These gene trees were then used to test for convergent rate shifts associated with
869 annual and perennial life histories and annuals as foreground clades, which can indicate repeated
870 selection. The procedure was done with 'RERconverge'.

871 The resulting p-values of both methods were corrected for multiple testing with the Benjamini-Hochberg
872 method (Benjamini and Hochberg 1995). If a gene showed corrected p-values ≤ 0.05 in both methods it
873 was considered a candidate gene putatively under divergent selection in annual and perennial *Hordeum*
874 species. Extraction of gene features and GO-term enrichment analysis were done the same way as
875 described for DE analysis above. The entire procedure was done twice, once with all accessions and once
876 without *H. bulbosum* accessions.

Data and resource availability

Raw data, a selection of intermediate results, supplements and scripts were stored in an ARC Data Hub (DOI: TBA).

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Authors' Contributions

TH: Conceptualization, Investigation, Methodology, Software, Formal analysis, Visualization, Writing - original draft, Data curation, Project administration. ND: Investigation. EBH: Methodology. MvK: Conceptualization, Funding acquisition, Resources, Writing - review & editing, Project administration, Supervision.

Supplementary Figures

Figure S1: Summary statistics of the read filtering process. Each dot represents one accession.

Figure S2: Schematic representation of the data creation workflow.

Figure S3: Phylogenetic tree of the unpruned set of accessions created by fasttree during the first OrthoFinder analysis. Accessions in red were removed due to their possible mislabeling.

Figure S4: Value of the six gene-partition properties against their sorted position. Order was determined according to their phylogenetic usefulness estimated by genesortR.

Figure S5: Pseudo-log-likelihood of phylogenetic network analysis using SNAQ averaged over repetitions (A) and per per number of reticulations (B-G) with networks below the threshold (green dotted line). Selected candidate networks are depicted in Fig. S6.

Figure S6: Candidate phylogenetic networks (A-H). Coloured lines indicate major (dark blue; $\gamma > 0.5$) and minor parent edges (light blue; $\gamma < 0.5$) of putative hybridization events. Their corresponding numbers represent the inheritance proportion (γ).

Figure S7: Maximum likelihood phylogenetic tree based on previously published genomes (designated as "genome accession tree" in the manuscript). To emphasize the branching order despite highly variable branch-lengths, the tree is rendered as a cladogram.

Figure S8: Species tree with mean (A) and 95% HPD interval (B) of divergence times as node labels.

Figure S9: Climatic niche spaces of individual Hordeum species separated by life-cycle strategy and estimated as n-dimensional hypervolumes.

Figure S10: Results from differential expression analysis of annual vs perennial accessions. Volcano plots (top row), heatmap of candidate genes (middle row; colours correspond to log 10(fold change) and graph of GO-term enrichment analysis (bottom row). Left column with and right column without *H. bulbosum* accessions. GO-terms are coloured according to p-values from yellow (high) to red (low).

Figure S11: Graph of GO-term enrichment analysis with (left column) and without (right column) *H. bulbosum* accessions. GO-terms are coloured according to p-values from yellow (high) to red (low).

Supplementary Tables

Table S1: List of plant material used for transcriptome assemblies.

Table S2: Pan-Hordeum consortion accessions used to complement the detection of orthologs

Table S3: Environmental variables of publicly available Hordeum observations after cleaning.

Table S4: Data sources of reference genomes used for creating the maximum likelihood phylogenetic tree based on previously published genomes(designated as "genome accession tree" in the manuscript; Fig. S7)

Table S5: Summary statistics of scHOG partitions inferred by genesortR.

Table S6: ABBA-BABA test results of selected candidate reticulations

Table S7: List of candidate genes from analyses of divergent genetic mechanisms and their assignment to eight major functional categories.

Table S8: List of orthogroups with output statistics of selection analysis without H. bulbosum accessions

Table S9: Gene copy number of each species and description of one representative H. comosum gene of each gene family that showed copy number variation between annual (red) and perennial (blue) Hordeum species.

Table S10: Members of gene families with divergent gene copy numbers between annual and perennial Hordeum species

Table S11: List of genes included in differential expression analysis without H. bulbosum accessions

Table S12: List of significant GO-terms of genes with significant signatures of selection with and without H. bulbosum accessions

Table S13: List of significant GO-terms of genes with significant signatures of selection with and without H. bulbosum accessions

Table S14: List of orthogroups with output statistics of selection analysis with H. bulbosum accessions

Table S15: List of genes included in differential expression analysis with H. bulbosum accessions

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