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. 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 cases of interspecific hybridization, three coinciding with major long-distance dispersal events across continents. Comparative climatic niche analysis indicated annual *Hordeum* species inhabit environments with higher temperatures, greater interannual variability, and increased human disturbance, compared to perennials, although no consistent precipitation differences were observed. Using our phylogeny as a framework, we analyzed selection and gene expression to uncover genomic changes associated with life-history strategy while accounting for phylogenetic non-independence. Additionally, we analysed gene copy number variations associated with life-history strategy. These analyses yielded 174 candidate genes across diverse biological functions, suggesting the genetic architecture underlying life-history evolution is more complex than assumed. Candidate genes grouped into six major functional categories, the most prominent being signal transduction and development, including regulators of flowering, dormancy, and meristem activity, metabolic and biosynthetic processes related to carbon allocation and storage, and stress response and defense, reflecting the resilience of perennials compared to the accelerated growth strategies of annuals. Our study reconstructs the evolutionary history and climatic niche differentiation of *Hordeum* species and demonstrates that convergent life-history evolution is driven by multifaceted, 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 et al.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 et al. 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 et al. 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 et al. 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.

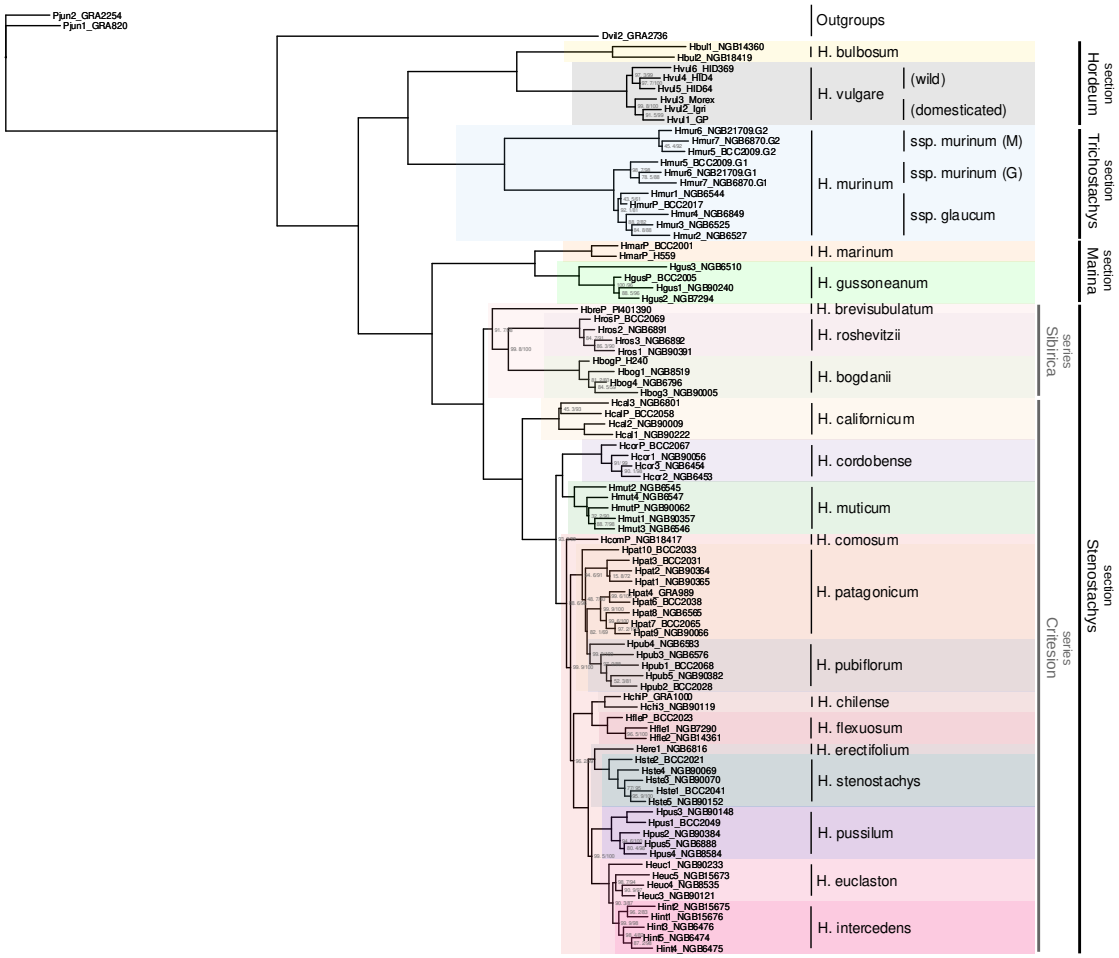
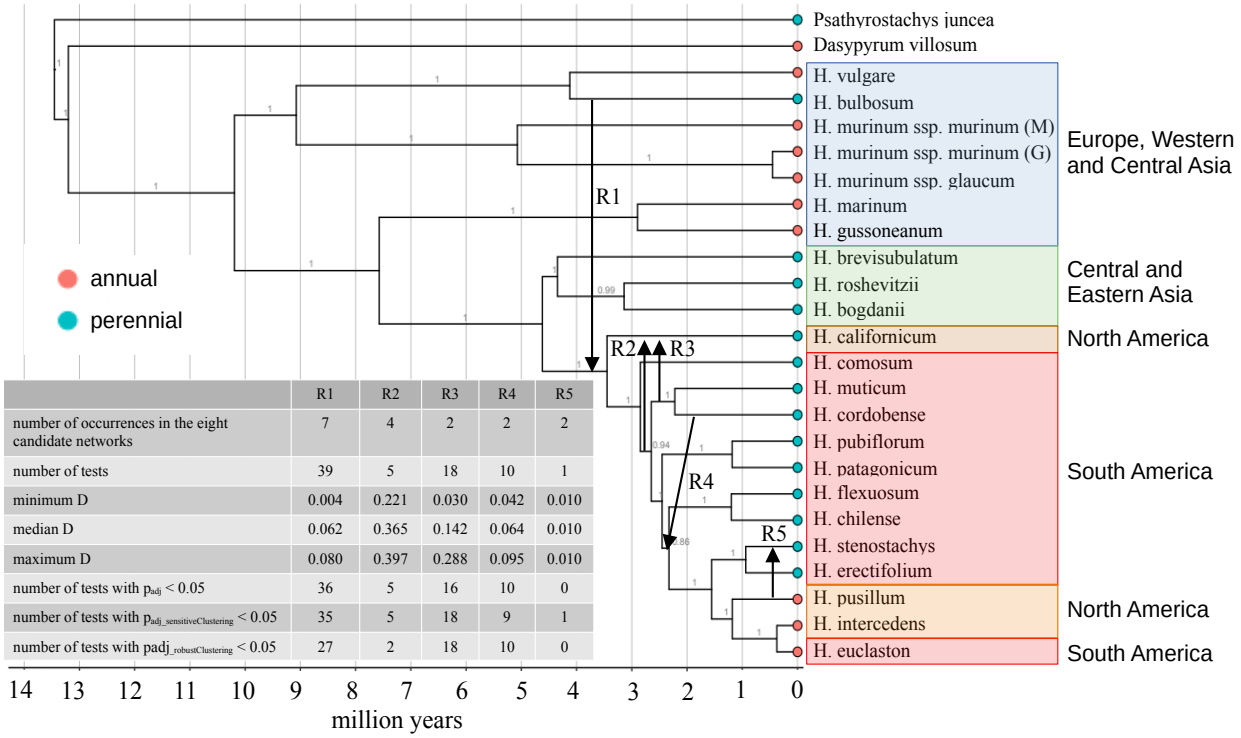


Figure 1: Accession based maximum likelihood tree. Node labels depict UFB/SH-aLRT values. Node labels are only shown if they were not the maximum of 100/100. Infrageneric groupings (sections and series) were defined sensu Blattner (2009).

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*.Transitions between life history strategies appear to have occurred independently on at least 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. It should be noted, however, that the exact branches along which the true transitions occurred cannot be inferred from the tree.



	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. 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.

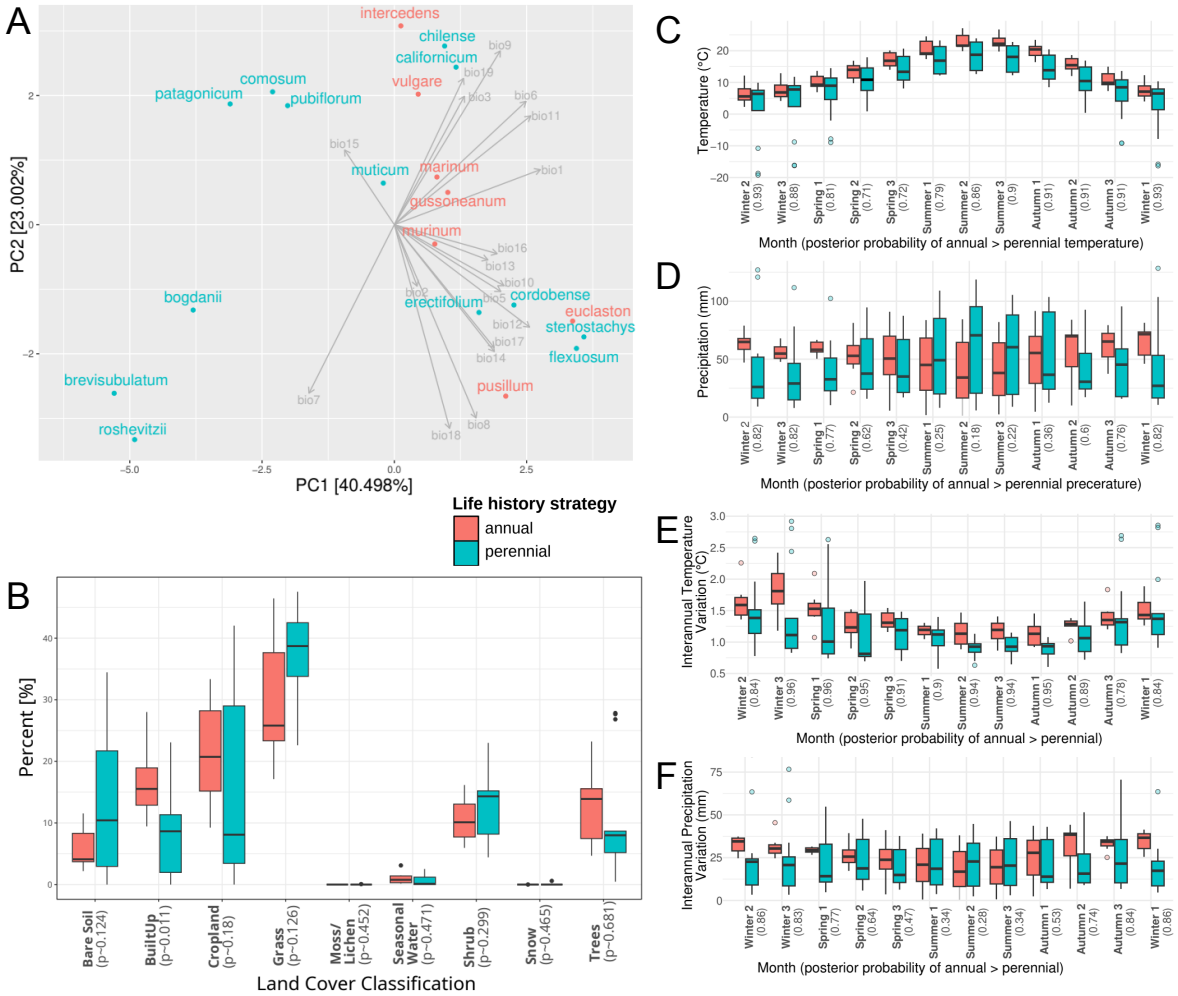


Figure 3: Habitat description of *Hordeum* species. A: Principal component analysis of species by bioclimatic variables. B: Boxplots of percentage landcover type of annual and perennial species with p-values from phylogenetic non-parametric ANOVA. C-F: Boxplots of monthly mean temperature (C), precipitation (D) and interannual variation of temperature (E) and precipitation (F) of annual and perennial species with posterior propabilities that annuals show higher values than perennials.

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 consistent differences in mean precipitation across entire seasons were apparent between the habitats of annual and perennial species; the strengh of evidence was month-specific. Posterior probabilities indicate moderate support for higher precipitation in annual habitats from late autumn to early-spring months ($P_{\text{annual > perennial}} \sim 0.76 - 0.82$; Fig. 3D). By contrast, summer months show moderate evidence for greater precipitation in perennial habitats ($P_{\text{annual > perennial}} \sim 0.18 - 0.25$, i.e. perennial > annual).

Temperature, however, tended to be higher in the habitats of annual species throughout the year. Posterior probabilities provide moderate to strong evidence that annuals experience higher temperatures in essen-

tially all months ($P_{annual > perennial} \sim 0.72–0.93$; Fig. 3C), with the strongest support from late summer through the winter months ($P_{annual > perennial} \geq 0.88$).

Analyses of interannual climatic variation produced a broadly similar picture for temperature variability: there was moderate to strong evidence that annuals occupy regions with greater interannual temperature variability with $P_{annual > perennial} \sim 0.78 - 0.96$ throughout the year (Fig. 3E). Interannual variation in precipitation shows weaker and more heterogeneous evidence: annual habitats show moderate evidence for greater interannual precipitation variability from late-autumn to early spring months ($P_{annual > perennial} \sim 0.74 - 0.86$; Fig. 3F), while the summer months exhibit no evidence for consistent differences between life histories.

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. 3B).

Taken together, differences in climatic niche size between annual and perennial *Hordeum* species are likely shaped by clade-specific adaptations. Meanwhile, climatic distinctions between their habitats were not characterized by a consistent year-round separation in precipitation, instead, posterior probabilities indicate a seasonally opposing pattern, with annual species occupying wetter habitats in winter, while perennial species tend to occur in wetter habitats during summer. These seasonal shifts contrast with the much clearer temperature signal. Annual habitats were consistently warmer throughout the year, with moderate to strong evidence for higher temperatures in all months, and they also showed markedly greater interannual temperature variability, particularly from late autumn through early spring. In line with the reduced temperature predictability of their environments, annual species also occurred more frequently in human-disturbed landscapes, which are known for lower ecological stability.

Divergent genetic determinants in annual and perennial *Hordeum* species

Understanding the genetic differences that distinguish annual from perennial Hordeum species can clarify the mechanisms underlying repeated transitions between these life-history strategies. We focused on three genomic features divergent between annual and perennial *Hordeum* species to uncover the genetic architecture (complexity) underlying the different life histories: (i) signatures of divergent selection in protein-coding sequences, (ii) differential gene expression, and (iii) gene copy number variation.

Across analyses of divergent selection, differential gene expression, and gene copy-number variation, we identified 174 candidate genes. Of these, 150 could be assigned to five broad functional categories - development, metabolism, cellular organisation, stress response, and transcriptional regulation - while 24 encoded proteins of unknown function or retrotransposon-related proteins (Tab. 2; Fig. 4A; Tab. S7). Because we derived functional assignments from conserved domains and annotations in *Arabidopsis thaliana* and other model plants, these categories should be considered general heuristics rather than precise descriptions of gene function in grasses or *Hordeum*. Candidate genes were distributed across all functional categories without significant enrichment (χ^2 tests, $p < 0.05$; Fig. 4A), indicating that divergence between annual and perennial life histories involves these functional categories equally. In line with this pattern, GO enrichment analyses identified terms largely corresponding to these five functional categories, including abiotic and biotic stress responses (oxidoreductase and hydrolase activities, nucleoside phosphate binding), metabolism (carbohydrate, hexose, small-molecule, vitamin, and protein metabolic processes), cellular organisation (microtubule-based movement, cellular component assembly), and transcriptional regulation (ribonucleotide and nucleotide binding; Tab. S12, S13).These results indicate that a complex genetic architecture covaries with repeated transitions between annuality and perenniality in

Hordeum. The following sections summarise the results from the selection, expression and gCNV analyses.

Table 2: Summary of the categorization of candidate genes across analyses of divergent genetic mechanisms; selection, differential expression and gene copy number variation (for a comprehensive list of candidate 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

To detect signatures of divergent selection between annual and perennial Hordeum species, we analyzed 2,908 scHOGs represented by at least two accessions per species using two complementary approaches: (i) D_n/D_s ratio modelling, and (ii) correlation between gene evolutionary rates and life-history strategy. A total of 576 genes showed significant Dn/Ds differences, and 442 showed significant trait-rate correlations. Applying a conservative criterion that required significance in both analyses, we identified 92 candidate scHOGs (Tab. S8). Most candidates (62) exhibited negative trait-rate correlations, indicating lower relative evolutionary rates in annuals and correspondingly higher D_n/D_s ratios in perennials. These genes thus appear to be under stronger selective constraints in annuals than in perennials. Because our analyses capture contemporary evolutionary rates rather than events associated with the original life-history transitions, the direction of historical transitions (~8–4.5 Myr from annual to perennial; ~1.5 Myr for the reverse; Fig. 2) is unlikely to influence these patterns. Nine genes showed the opposite pattern, with higher

evolutionary rates and higher Dn/Ds ratios in annuals. The remaining 20 genes displayed conflicting signals between the two methods, typically cases where one statistic was significant but effect sizes were small (e.g., RNA.HOG0025477). These genes may represent true candidates, but their support is weaker relative to the core set.

To quantify expression divergence between annual and perennial accessions, we analyzed pooled RNA-seq data from leaves, roots, stems, and inflorescences, which provided 7,942 scHOGs with adequate read counts. A generalized linear mixed model incorporating phylogenetic relatedness identified 41 DEGs at $p_{adj} \leq 0.01$ and $|\log_2 FC| \geq 1$ (Fig. 4C; Tab. S11; Fig. S10). Of these, 25 DEGs were on average more highly expressed in annuals, and 16 were more highly expressed in perennials. Variation analyses showed that 20 genes had >10% higher expression coefficient of variation (CV) in annuals, 8 genes had higher CV in perennials, and 13 showed similar variability in both life-history groups The pattern of higher mean expression and greater variability in annuals appears consistent with their fast-paced, resource-intensive life strategy, which may require both elevated and flexible gene regulation, in contrast to the more stable and regulated expression profiles of long-lived perennial.

To examine potential genomic dosage differences, we tested 29,565 gene families for consistent copy-number differences between life histories. We identified 41 gene families in which all perennial species across the different branches of the phylogeny possessed equal or higher copy numbers than annuals (Tab. S9). No families showed the reverse pattern. Among these, ten families had a single copy in annuals but between two and nine copies in perennials (e.g., GENO.HOG0004489, GENO.HOG0005530; Tab. S9). Another eight families were absent in annuals but present in one or more copies in perennials. More than half of these gene families fell into the "Mobile Genetic Elements & Unknown Functions" category, reflecting the high propensity of transposable elements to undergo copy-number expansion. To further investigate if these copies were also expressed, we examined gene expression for each gene copy within these families. In six families, perennials expressed more gene copies in our data than were present in annuals. Notable examples include GENO.HOG0004711, a senescence regulator, and GENO.HOG0007793, a TEOSINTE BRANCHED1-like gene, 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). We note that these expression results are conservative, as multimapping reads were excluded. Some paralogs may also be expressed only under specific developmental or environmental contexts not represented in our sampling. Nonetheless, these findings demonstrate consistent gCNV differences between life histories and establish a basis for more detailed investigations into whether this variation affects expression, alters genomic dosage, or supplies raw material for evolutionary innovations such as neo-functionalization.

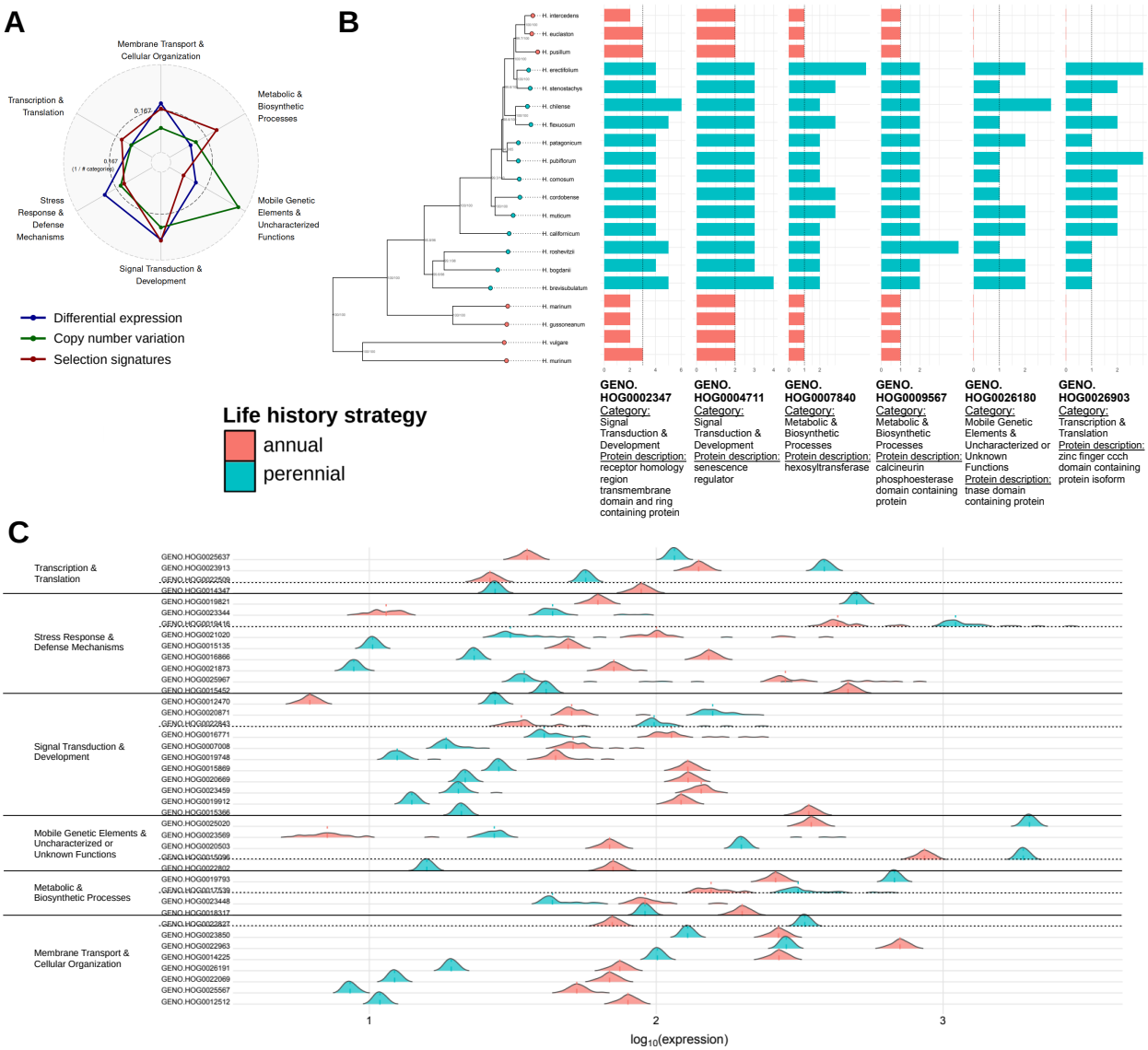


Figure 4: Divergent genetic mechanisms in annual and perennial Hordeum species without *H. bulbosum*. A: Radar plot of the number of genes per analysis assigned to categories. Numbers are relative to the size of the analyses. B: The genome accession tree excluding outgroup species (left). Node labels 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. S9 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.

Although no single gene was identified by more than one analytical approach, candidate genes from all analyses converged on broadly similar functional categories (Tab. S7).

The largest group fell into Signal 'Transduction & Development', comprising 45 of the 92 candidates. These genes include regulators of meristem maintenance, organ boundary formation, bud dormancy, and flowering-time control, all prime regulators of life-history strategies. Examples include *SCARECROW-LIKE6* (RNA.HOG0015866), involved in radial patterning and stem cell maintenance; a LOB-domain protein (GENO.HOG0022843), associated with boundary specification; and several NAC-domain transcription factors (GENO.HOG0031918, GENO.HOG0020669, RNA.HOG0010144), which influence both meristem function and senescence regulation (Bolle 2004; Curaba et al. 2013; Xing et al. 2024; 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 net-

works that control cell division, differentiation, and organ patterning in response to both intrinsic and extrinsic signals (Dubos et al., 2010; Ramsay & Glover, 2005).

A second group of candidates belonged to 'Transcription & Translation', including chromatin remodeler orthologs such as RNA.HOG0026351 (orthologous to *AtSKIP*), RNA.HOG0027556 (orthologous to *AtPIE1*), and RNA.HOG0028859 (orthologous to *AtSWP73*). 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 candidate genes fell into the 'Metabolic & Biosynthetic Processes' category, encompassing carbohydrate metabolism, carbon allocation, and cell-wall biosynthesis and remodeling. Several genes encode glycosyltransferases (GENO.HOG0007840, RNA.HOG0026057, RNA.HOG0010428), which catalyze sugar-nucleotide–dependent reactions important for carbohydrate modification. Others, including invertases (GENO.HOG0002011, GENO.HOG0008038) and pyrophosphate–fructose-6-phosphate phosphotransferase (RNA.HOG0011119), are involved in carbon partitioning between growth and storage tissues (van der Merwe et al. 2010; Duan et al. 2016). Additional candidates, such as 4-coumarate-CoA ligase, glycerophosphodiester phosphodiesterase, glycosyl hydrolases, pectate lyases, and glucan endo-β-glucosidases, participate in cell-wall formation and remodeling, which can affect structural and metabolic properties of tissues. While the precise roles of these genes in annual–perennial differences remain to be determined, their functions point to potential variation in carbohydrate processing, carbon allocation, and cell-wall biosynthesis, processes that may underlie differences in growth strategy and resource management between life-histories strategies.

The 'Stress Response & Defense' category (27 candidates) included AP2/ERF transcription factors strongly upregulated in annuals (GENO.HOG0021020, GENO.HOG0021873), orthologous to *AtERF105* and *AtERF53*, which mediate cold tolerance, ABA signaling, and heat responses (Hsieh et al. 2013; Illgen et al. 2020; Ma et al. 2024). Perennials, by contrast, showed higher expression of genes involved in oxidative stress mitigation, such as flavin-containing monooxygenases (GENO.HOG0016866, GENO.HOG0007274) and chalcone synthase (GENO.HOG0019416), which contributes to flavonoid biosynthesis and antioxidant defense (Dao et al. 2011). These contrasting signatures suggest that annuals invest more heavily in acute stress-responsive transcriptional activation, whereas perennials rely on constitutive or longer-term protective pathways consistent with multi-year survival.

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 et al. 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 et al. 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 et al. (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 et al. 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 et al. 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 et al. 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 exhibited distinct seasonal climatic patterns in their habitats. In winter, habitats of annual species were both warmer and wetter than those of perennials, likely promoting vegetative growth and seedling establishment. In contrast, during summer, annual habitats were hotter and drier, possibly imposing terminal stress that favours senescence and survival in the seed stage, a typical annual-perennial ecological pattern (Stearns 1992). This seasonal contrast in temperature and precipitation is consistent with classical life-history theory for grasses: accelerated development and early maturation are common escape strategies of grasses facing potentially terminal stress (Loka et al., 2019; Jagadish, 2020; Bahrami et al., 2021).

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 temperatures and winter precipitation 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 transition between annual and perennial life history 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 and genes (Friedman and Willis 2013; Heidel et al. 2016 ; Kiefer et al. 2017; 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). While studies in the Brassicaceae suggest that genetic changes in a limited number of developmental genes can drive transitions along life-history strategies (Zhai et al. 2024), candidate genes differentiating between annual and perennial *Hordeum* species spanned a broad array of functions. However, from our analysis we cannot distinguish between genes that are directly causal for differences in plant longevity and those that more likely covary with life history variation, such as biotic and abiotic stress resistance genes. Our data sug-

gests that the genetic basis of perennialism involves a suite of changes in plant developmental, physiology, nutrient allocation and defense in *Hordeum*, suggesting that 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 genetic modifications in many functions and pathways.

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 be 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.

Material and Methods

Plant Material and RNA sequencing

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

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

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

Transcriptome assemblies

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

Transcripts were assembled *denovo* using rnaSPADES (v3.9.1; Bushmanova et al., 2019; Prjibelski et al., 2020) with default settings. Additionally, a genome-guided assembly was done with Trinity (v2.2.0; Grabherr et al., 2011). We ran Trinity three times with kmer 20, 26 and 32 and the parameters --SS_lib_type RF and --genome_guided_max_intron 2500. The bam files required for genome-guided assembly were created by mapping the filtered reads against the reference genomes of the respective species (Tab. S1) except for the outgroup *Psathyrostachys juncea* (Fisch.), which due to the lack of a

636 reference genome, was mapped against the reference of the second outgroup *Dasypyrum villosum* (L.
637 Borbás; X. Zhang et al. 2023). Our collection contained two tetraploid species, *Hordeum bulbosum* (L.;
638 autotetraploid) and *Hordeum murinum* ssp. *murinum* (L.; allotetraploid). For autotetraploid *H. bulbosum*
639 we arbitrarily chose genome 1 from the reference genome and treated the two accessions as diploids.
640 Reads from *H. m.* ssp. *murinum* accessions were mapped against the allotetraploid genome. Reads that
641 mapped to genomes 1 and 2 (herein denoted as 'G' and 'M', respectively, *sensu* Rajhathy & Morrison,
642 1962) were extracted and separately mapped against the separated G or M genome reference. The
643 separated reads from the G and M genome of each *H. m.* ssp. *murinum* accessions were treated like two
644 diploid samples for further procedure. The mapping procedure was done using STAR (v2.7.10a; Dobin et
645 al., 2013) with default settings. In a subsequent filtering step with SAMtools (v1.3.1; Danecek et al.,
646 2021), only uniquely mapped reads were retained.

647 The four assemblies per accession were merged into a single fasta file and mapped against the same
648 reference genome used for read mapping using Minimap2 with the '-ax splice' option (v2.24; H. Li,
649 2018). The longest transcript was extracted from the resulting bam file with AGAT (v0.8.0; Dainat,
650 2020). We used BLAST+ (v2.2.26; Camacho et al., 2009) with the parameters -max_target_seqs 1, -
651 outfmt 6 and -evalue 1e-5 to create a database based on the concatenated proteins of the barley variety
652 Morex (v3; Mascher et al., 2021) and *D. villosum*. This database was input for Transdecoder (v5.5.0;
653 Haas, 2024) which we used with default parameters to predict open reading frames and collections of the
654 longest transcript, coding and protein sequences.

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

656 **Detection and processing of orthologs**

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

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

674 After running OrthoFinder again with the pruned set of accessions, we extracted single-copy hierarchical
675 orthogroups (scHOGs) which were present in all accessions and aligned their coding sequences to create a
676 multiple sequence alignment (MSA) for each scHOG. Alignment was done with MAFFT (v7.407; Katoh
677 et al., 2002). Each MSA was trimmed using Clipkit (v1.4.1; Steenwyk et al., 2020) with default
678 parameters to increase the signal-to-noise ratio and improve the MSAs' quality for subsequent
679 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.

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).

727 **Interspecies hybridization**

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

733 Phylogenetic network inference was conducted with the maximum pseudo-likelihood approach developed
734 for SNAQ (Solís-Lemus et al. 2017) and implemented in the Julia (v1.10.2; Bezanson et al., 2017)
735 package 'PhyloNetworks' (v0.16.3; Solís-Lemus et al., 2017). Genetrees from the aligned coding
736 sequences of the two-accessions set were estimated with IQtree (v2.2.0; Minh et al., 2020) using the same
737 procedure as outlined above for creating the input of genesortR (Koch 2021). Species quartet concordance
738 factors were calculated from these genetrees. We ran SNAQ 10 independent times with different seeds,
739 allowed reticulations ranging from 0-10 and the species tree as a guide tree. We considered all networks
740 with a pseudo log-likelihood below a certain threshold as candidate networks. This threshold was
741 arbitrary. Yet, in all models, there was a steep increase in pseudo-log-likelihood between networks below
742 and above this threshold, suggesting a clear decrease in statistical support for networks above the
743 threshold (Fig. S5). Reticulations that appeared in at least two networks were considered candidate
744 reticulations (Fig. S6).

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

761 Linkage disequilibrium (LD) pruning was done with PLINK (v 1.90b7; Purcell et al., 2007). Windows of
762 50 kb were shifted by five variants, and SNPs with $r^2 \geq 0.5$ within one window were removed. The pruned
763 SNP set was used as input for Dsuite (v0.5r53; Malinsky et al., 2021) to conduct ABBA-BABA tests for
764 candidate reticulations found with SNAQ (Solís-Lemus et al. 2017). In all tests, *Dasypyrum villosum*
765 was the outgroup species. In Dsuite, we used the --ABBAclustering parameter to test whether ABBA
766 patterns in tests with significant D tend to cluster together (Koppetsch et al. 2024).

767 **Biogeography of annual and perennial *Hordeum* species**

768 We downloaded publicly available georeferenced observations of the diploid *Hordeum* species (excluding
769 domesticated barley) included in our phylogeny from GBIF (GBIF.org 2022), the New York Botanical
770 Garden (NANSH; http://sweetgum.nybg.org/science/ih/; accessed 16.08.2022) and the North American
771 Network of Small Herbaria (https://nansh.org/portal/collections/index.php; accessed 16.08.2022). Since
772 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. Because the timing of growth seasons differs between hemispheres, we classified spring, summer, autumn, and winter according to the conventional meteorological months for the Northern and Southern Hemispheres, respectively. In the Northern Hemisphere, spring was represented by March-May, summer by June-August, autumn by September-November, and winter by December-February, whereas in the Southern Hemisphere these groupings were shifted by six months (e.g., spring: September-November). This procedure ensured that seasonal climate descriptors match major growth cycle patterns across both hemispheres.

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.

We modeled monthly temperature and precipitation variation between annual and perennial species using Bayesian generalized additive mixed models (GAMMs) implemented in the R package 'brms' (Bürkner, 2017). Temperature/precipitation was the response variable, and life history (annual vs. perennial) was included as a fixed factor. Smooth functions of month were used to capture cyclic seasonal patterns; i.e. each month was coded numerically (1-12) to represent the annual cycle, with consecutive numbering from January (winter) to December, and treated as a cyclic variable. This ensured that December and January were modeled as adjacent months. Life-history-specific smooths allowed the two groups to differ in their seasonal temperature profiles. The model included a phylogenetic random effect based on the correlation matrix derived from the species tree to account for non-independence due to shared ancestry. Model convergence was evaluated using standard diagnostics implemented via the 'brms' package. Posterior predictions were generated for all months and both life histories using species-averaged estimates. For each month, we calculated the posterior probability that the predicted climate value for annual species exceeded that for perennials, P(annual > perennial), and interpreted these probabilities as measures of evidence for systematic differences between life histories. Since no formal thresholds exist, we used the following guideline: values between 0.50 and 0.75 were considered to indicate no evidence, values from 0.75 to 0.90 moderate evidence, and values above 0.90 strong evidence that annuals exceed perennials. Because the scale is symmetric, probabilities below 0.50 provide evidence in the opposite direction, with 0.50-0.25 indicating no evidence, 0.25-0.10 moderate evidence, and values below 0.10 strong evidence that perennials exceed annuals.

To quantify interannual climate variability across the habitats of annual and perennial *Hordeum* species, we obtained monthly precipitation and minimum and maximum temperature data for the period 1960–2019 (Fick and Hijmans 2017). For each month, the mean of the minimum and maximum temperatures was used as a summary measure of monthly temperature. Interannual variability in temperature and precipitation was estimated as the median absolute deviation across years for each month. Bayesian GAMMs were then fitted, following the same procedures described above, to test for differences in interannual climatic variability between life-history strategies.

821 Differences in landcover between annuals and perennials were tested using the non-paramtetric phylogen-
822 etic ANOVA implemented in the R package 'phytools' (v2.5-2) with the species tree to model ancestral
823 covariance (Revell, 2024).

824 The entire procedure was done in R (v4.3.3; R CoreTeam, 2021).

825 As mentioned above, we focused on iteroparous species that continuously maintain above-ground veget-
826 ative material and, therefore, excluded the pseudo-annual *H. bulbosum* from this analysis.

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

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

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

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

847 A gene family was considered a candidate if the gene count of the smallest family in an annual species
848 exceeded the gene count of the largest family in a perennial species or vice versa. The procedure was
849 done in R (v4.3.3; R Core Team, 2021) using base functions. Gene features including GO-terms of these
850 final candidate genes were extracted from the *Hordeum comosum* accession (NGB18417) for consistency
851 with expression and selection analyses (see below). Gene features and GO-terms were extracted from one
852 representative gene per family. No GO-term enrichment analysis was conducted for copy number
853 candidates because only eight candidates had GO-term annotatins.

854 To be comprehensive, we also conducted the analysis a second time with *H. bulbosum* included. Yet,
855 these results are not discussed and are only presented in the supplementary files. The same procedures
856 were followed for the analyses of differential expression and selection explained below.

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

858 For the analysis of differential gene expression, only diploid *Hordeum* accessions subjected to RNA
859 sequencing were used. Read counts were derived from the mapped reads using HTSeq (v2.0; Putri et al.,
860 2022). Gene counts of accessions were sorted according to assignments to scHOGs done by OrthoFinder
861 (v2.5.4; Emms & Kelly, 2019) for the analysis of gene copy number variation described above. Genes'
862 tagwise dispersion and size factors (using "TMM") were estimated with the R package 'edgeR' (v4.2.2;
863 Chen et al. 2025). Significant differential expression between annual and perennial accessions were tested

by fitting a generalized mixed linear model with negative binomial errors and a log link function (ngGLMM) which was implemented the R package 'glmmSeq' (v.0.5.5; Lewis et al., 2024). The phylogenetic relationship among accessions was included as random effect variables to account non-independence of accessions. The phylogenetic relationship was determined by the first three principal coordinates of the cophenetic distances of the ML based accession tree from the 87 pruned set of accessions while excluding the 13 accessions from Feng et al.(2025), because they were not subjected to RNA sequencing. P-values were corrected for multiple testing with the Benjamini-Hochberg method (Benjamini and Hochberg 1995). Genes were considered differentially expressed (DE) candidates if their corrected p-value was ≤ 0.01 and their $|\log_2(\text{fold change})|$ was ≥ 1.

Gene features including GO-terms of these final candidate genes were extracted from the *Hordeum comosum* accession (NGB18417) because it was the only accession that had a sequence in all scHOGs of the two-accession set and available gene features from Feng et al. (2025). GO-term enrichment analysis was done for biological processes using a Fischer‘s exact test with all genes used for the analysis as background and candidate genes as genes of interest. The analysis was done in the R package ‘topGO’ (v2.56.0; Rahnenfuhrer & Alexa, 2024). GO-terms were considered significant if the p-values were $\leq$ 0.05. The entire procedure was done twice, once with all accessions and once without *H. bulbosum* accessions.

Selection signatures in the context of life-history strategies

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

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

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

Data and resource availability

Raw data, a selection of intermediate results, supplements and scripts were stored in the DataPlant Hub https://git.nfdi4plants.org/timo.hellwig/evolution_and_divergent_genetic-mechanisms_of_annual_and_perennial_hordeum_species.

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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).

941 **Supplementary Tables**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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