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Adaptive Genomic Divergence Across Altitudes in *Capsella bursa-pastoris*

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ABSTRACT

Understanding how natural selection drives local adaptation is fundamental to evolutionary biology. *Capsella bursa-pastoris*, a close relative of *Arabidopsis thaliana*, is widely distributed across diverse habitats worldwide. However, the genetic basis underlying its local adaptation remains unclear. Here, we analyzed 40 individuals from 14 populations across China using population genomic approaches to investigate genetic structure and adaptive selection. Our results revealed a clear genomic divergence between western high-altitude and eastern low-altitude populations of *C. bursa-pastoris*. We identified 54 candidate genes under positive selection associated with altitudinal divergence, including energy metabolism, photosynthesis, signal transduction, growth regulation, and membrane transport. Most of these genes were located in subgenome CbpB, suggesting that this subgenome harbors an enrichment of loci potentially involved in adaptive differentiation. Moreover, genotype–environment association and partial redundancy analyses highlighted a strong influence of ecological factors on genetic variation. Notably, loci associated with environmental variables were primarily linked to responses to light, temperature, and water availability, indicating their likely roles in local adaptation. Overall, our findings indicate that ecological selection is an important driver of population divergence in *C. bursa-pastoris* and provide a robust genomic framework for understanding its adaptation across heterogeneous environments.

1 | Introduction

Spatial variation in environmental conditions can generate heterogeneous selection pressures, driving local adaptation and genetic differentiation among populations. Local adaptation occurs when populations evolve higher fitness in their native environments than in others within the species' range [1–3]. It plays a central role in shaping evolutionary trajectories, species distributions, and ecological resilience under changing climates. Understanding how natural selection shapes patterns of genetic variation across environments is therefore a key goal in

evolutionary biology. Over the past decades, increasing attention has been devoted to uncovering the genetic basis of adaptive divergence, with many studies revealing that populations often exhibit fine-scale adaptation to their local environments in response to spatially variable selective pressures [4–9]. Plants inhabiting high-altitude environments are exposed to a range of abiotic stresses, including low temperatures, intense ultraviolet (UV) radiation, hypoxic conditions, and limited water availability. To cope with these extreme conditions, they have developed complex adaptive mechanisms underpinned by genetic variation

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Highlights

- Genomic divergence was observed between high-altitude and low-altitude populations of *Capsella bursa-pastoris* in China, consistent with an important role of ecological factors.
- Candidate adaptive loci associated with enhanced energy metabolism, photoprotection, and growth plasticity under altitudinal stress were identified.

[10–12]. High-altitude plants frequently exhibit significant levels of genetic polymorphism across populations, reflecting long-term natural selection and local adaptation to different altitudinal niches. Such polymorphisms can affect coding and regulatory regions, leading to functional diversity in traits that are critical for survival in high-elevation ecosystems.

Capsella bursa-pastoris (L.) Medik. is a close relative of *Arabidopsis thaliana* and is a predominantly selfing tetraploid species with disomic inheritance [13]. It is a summer to winter annual with great colonizing ability and a nearly worldwide distribution [14]. It is now widely accepted that the species originated from the hybridization, ca. 100,000 to 300,000 years ago, of the ancestral lineages of two diploid species of the same genus, *Capsella orientalis* Klokov and *Capsella grandiflora* (Fauché & Chaub.) Boiss. [15, 16]. *C. bursa-pastoris* has been shown to originate in Eurasia, either from the Middle East or Europe (not yet certain), followed by subsequent invasion into Asia [17]. The recent worldwide spread of the species was likely associated with human migrations [13, 17]. In a large-scale experiment involving two common gardens located in Europe and Asia and 232 *C. bursa-pastoris* accessions from 59 sites distributed across Europe, Asia, North Africa, and the Middle East, local adaptation was not observed; instead, a relatively high level of phenotypic plasticity was detected to be essential for the successful rapid range expansion of the species [18]. However, since a successful expansion normally corresponds to a particular combination of phenotypic plasticity and genetic adaptation, which are not mutually exclusive [19], the finding in Cornille et al. [18] is probably due to the large geographical scale considered, and thus differentiation at a smaller geographical scale merits further investigation.

Asian *C. bursa-pastoris* populations have reduced genetic diversity and a higher number of deleterious mutations than populations from the Middle East and Europe, suggesting a much higher genetic load in Asia [16, 20]. Despite their extremely low genetic diversity, Asian populations experience highly variable environmental conditions. In China, *C. bursa-pastoris* populations occur across a broad array of complex environments, ranging from subtropical climatic conditions in the south to arid/semiarid environments in the northwest and boreal climates in the northeast. Similarly, *C. bursa-pastoris* populations are distributed from the eastern low-altitude coast to the western Qinghai–Tibetan Plateau with elevations over 4000 m above sea level. Therefore, *C. bursa-pastoris* in China provides an outstanding opportunity to examine local adaptation, such as high-altitude adaptation, in a recently introduced

allopolyploid [17]. Here, we resequenced 40 *C. bursa-pastoris* accessions from 14 populations in China; 7 of these populations were sampled from high-altitude regions, and the remaining 7 from low-altitude areas. With the recent publication of a high-quality reference genome for *C. bursa-pastoris* [21], we were able to perform comprehensive analyses of genetic variation across these accessions. The aim of this study is to investigate the population genetic structure of these *C. bursa-pastoris* accessions and to uncover the genetic basis underlying adaptation to high-altitude environments. Ultimately, we seek to gain a better understanding of how *C. bursa-pastoris* responds to altitudinal variation, providing insights into the evolutionary mechanisms that shape local adaptation in this widespread species.

2 | Materials & Methods

2.1 | Sample Collection, DNA Extraction, and Sequencing

We selected 40 *C. bursa-pastoris* accessions from 14 populations (2–3 accessions per population) in China, due to the extremely low genetic diversity observed in Asian populations [16, 17, 22]; 7 of these populations were sampled from high-altitude regions, and the remaining 7 from low-altitude areas (Supporting Information S2: Table S1). The geographic information of the populations was determined using GPS. Seeds were stratified on moist filter paper for a week at 4°C and subsequently transferred to soil in pots, which were placed in randomized positions in a growth chamber at 23°C under a 16 h light/8 h dark photoperiod. Genomic DNA was extracted from silica gel-dried leaf samples using a modified CTAB protocol [23]. After assessing DNA quality and quantity by electrophoresis and Qubit 2.0, DNA was shipped to Novogene Bioinformatics Institute (Beijing, China) for library preparation and sequencing. Paired-end 150-bp reads were generated using Illumina NovaSeq 6000 systems (San Diego, CA, USA). All sequences were submitted to the National Center for Biotechnology Information's Sequence Read Archive and the China National Center for Bioinformation's Genome Sequence Archive (BioProject ID: PRJNA1337124 and PRJCA047565; Supporting Information S2: Table S2).

2.2 | Alignment and Variant Calling

Sequence read quality was assessed with FASTQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc>). Adapter sequences and low-quality bases were removed using TRIMMOMATIC [24]. All clean reads were then mapped to the recently published *C. bursa-pastoris* reference genome [21] using the BWA-MEM algorithm with default parameters [25]. Potential PCR duplicates were marked using Picard Tools 2.23.3 (<https://github.com/broadinstitute/picard>) and were removed during subsequent variant calling. Variant calling was performed using HaplotypeCaller from the Genome Analysis Tool Kit (GATK) v 4.1.9.0 [26]. GenotypeGVCFs was then used to construct multi-sample joint aggregation and genotype likelihood correction. Variants were filtered to retain only biallelic SNPs (Single Nucleotide Polymorphisms). GATK hard filters were set to QD < 2.0, FS > 60.0, SOR > 3.0, MQ < 40.0, MQRankSum < -12.5, ReadPosRankSum < -8.0, and ReadPosRankSum

> 8.0. Finally, we filtered out sites with minor allele frequency (MAF) < 5% or missing rate > 10%, and performed a linkage disequilibrium (LD)-based SNP pruning process in PLINK v1.90 (option--indep-pairwise 50 10 0.2; [27]) to exclude strongly linked SNPs. A MAF threshold of 5% was chosen given the relatively small sample size (40 individuals), which helps to remove rare variants that may introduce noise while retaining sufficient informative SNPs for downstream analyses [28, 29]. The LD-pruning parameters followed commonly used practices to balance the removal of linked SNPs with maintaining a representative set of independent markers [28, 30]. SnpEff [31] was used to annotate and predict the effects of the identified SNPs. Genome-wide and subgenome-specific nucleotide diversity (π) were calculated as SNP-weighted means across 50-kb windows using VCFtools [32].

2.3 | Population Genetic Structure

To investigate the genetic structure of *C. bursa-pastoris*, three methods were used based on 1,628,822 genome-wide SNPs. First, AdmixPipe v3.2.1 [33], a wrapper pipeline for the program Admixture v1.3.0 [34], was employed to infer ancestral components. Admixture was run with K values ranging from 2 to 10, and the optimal number of K was determined by the cross-validation (CV) error, selecting the K with the lowest value. Then, principal component analysis (PCA) was performed using the R package SNPRELATE [35] to visualize genetic differentiation among populations. Based on altitude classifications, that is, high altitude (2656–4140 m) and low altitude (13–100 m), we divided the 14 populations into two altitude categories for downstream comparison. Finally, phylogenetic relationships were reconstructed among populations using IQ-TREE v2.3.6 [36], with two individuals from Sweden (SE33) and Jordan (JO56) as outgroups. The best-fit substitution model was selected using ModelFinder [37] under the Bayesian Information Criterion (BIC) score. Branch support was assessed using both SH-like approximate likelihood ratio test (SH-aLRT) and ultrafast bootstrap (UFBoot) with 1000 replicates.

2.4 | Pairwise Genetic Differentiation and Selection Sweeps

To investigate genome-wide signatures of altitudinal adaptation, VCFtools was first used to calculate pairwise genetic differentiation (F_{ST}) between high- and low-altitude groups in sliding windows across the genome, with a window size of 10,000 bp. The windows with F_{ST} values in the top 1% of the empirical distribution were defined as outliers. We also conducted a genome-wide scan using XP-CLR [38], which computes cross-population composite likelihood ratio (XPCLR score), a composite evaluation test based on multilocus allele frequency differentiation between two groups. Higher XPCLR scores indicate stronger shift of allele frequency spectrum and extended LD, which are indicative of positive selection. Normalized XPCLR scores were calculated in a sliding window of 10,000 bp with a step size of 10,000 bp, containing 2 to 50 SNPs per window. Genomic regions with normalized XPCLR scores exceeding the 99% quantile (score > 4.86) were defined under selection. Finally, windows identified as outliers in both the F_{ST} and XP-CLR analyses were considered to represent genomic regions potentially under divergent selection between high- and low-

altitude populations. Genes located within these outlier regions were considered candidate genes potentially involved in altitudinal adaptation. KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis was subsequently performed on these candidate genes to identify biological pathways associated with high-altitude adaptation [39], and false discovery rates (FDR) were calculated using the Benjamini–Hochberg (BH) method.

2.5 | Genotype–Environment Associations and Landscape Genomic Analysis

To investigate the impact of climate, geographic location and genetic structure on the genetic variation in *C. bursa-pastoris* populations, we used R package “vegan” [40] to conduct genotype–environment association and landscape genomic analyses. Climatic variables were obtained from the WorldClim database (19 bioclimatic variables at 30 arc-seconds resolution) using the geographic coordinates of each population. Prior to analysis, environmental variables were pre-processed by excluding those with no explanatory contribution and by removing highly correlated variables ($|r^2| > 0.9$). We first constructed a full redundancy analysis (RDA) model, in which genetic data (allele frequencies at SNP loci) were used as the response variable, and climate variables, geographic coordinates (longitude and latitude), and genetic structure (represented by principal components) were simultaneously included as explanatory variables. Subsequently, partial RDAs (pRDA) were performed to partition the effects of each factor by controlling for the influence of the others, thereby estimating the unique contributions of the individual climate, geographic, and population structure models to genetic variation. Based on these seven models, we further calculated the proportion of variance (adjusted R^2) using the function “RsquareAdj” and tested model significance with “anova.cca”.

To identify loci potentially associated with environmental gradients, we applied the “rdadapt” function to partial climate models and selected the top 1% of SNPs most strongly associated with climatic variables. We visualized the relationships between selected climatic factors and the first two RDA axes (RDA1 and RDA2). Finally, genes harboring highly associated SNPs were subjected to KEGG and GO (Gene Ontology) functional enrichment analyses using TBtools [41].

3 | Results

3.1 | Genome Sequencing and SNP Calling

In order to detect population genetic structure and adaptive signatures between high- and low-altitude *C. bursa-pastoris*, we first generated genomic Illumina sequence data from 40 accessions. The major characteristics of these libraries are summarized in Supporting Information S2: Table S2. In total, nearly 279.79 Gb of genomic data were obtained, ranging from approximately 5.26–9.78 Gb for each library, corresponding to c. 15.94× to 29.64× coverage mapped onto the *C. bursa-pastoris* reference. A total of 1,631,247 high-quality SNPs were retained across these accessions after variant calling and subsequent stringent filtering. The genome-wide nucleotide diversity (π) was estimated at 0.00287, with comparable levels of

diversity observed in subgenomes CbpA ($\pi = 0.00292$) and CbpB ($\pi = 0.00281$).

3.2 | Population Structure

Based on the Admixture analysis, we identified two major genetic lineages of *C. bursa-pastoris*, with the CV-error reaching its minimum at $K = 2$ (Figure 1b, Supporting Information S2: Figure S1). One lineage corresponds to the low-altitude populations (DW, JA, BL, CSH, XY, AQ, and HF), and the other to

the high-altitude populations (LJH, CJ, TA, SP, HUY, ZD, and LL). Notably, five populations (JA, XY, AQ, HF, and LJH) exhibited mixed ancestral components, suggesting gene flow between the two lineages. This structure was further supported by PCA, which clearly separated individuals into two groups (Figure 1c). The phylogenetic tree showed that the high-altitude lineage formed a monophyletic clade, while the low-altitude lineage was paraphyletic and located closer to the root of the tree (Figure 1d). Although XY and HF are located in low-altitude regions, they cluster genetically with the high-altitude

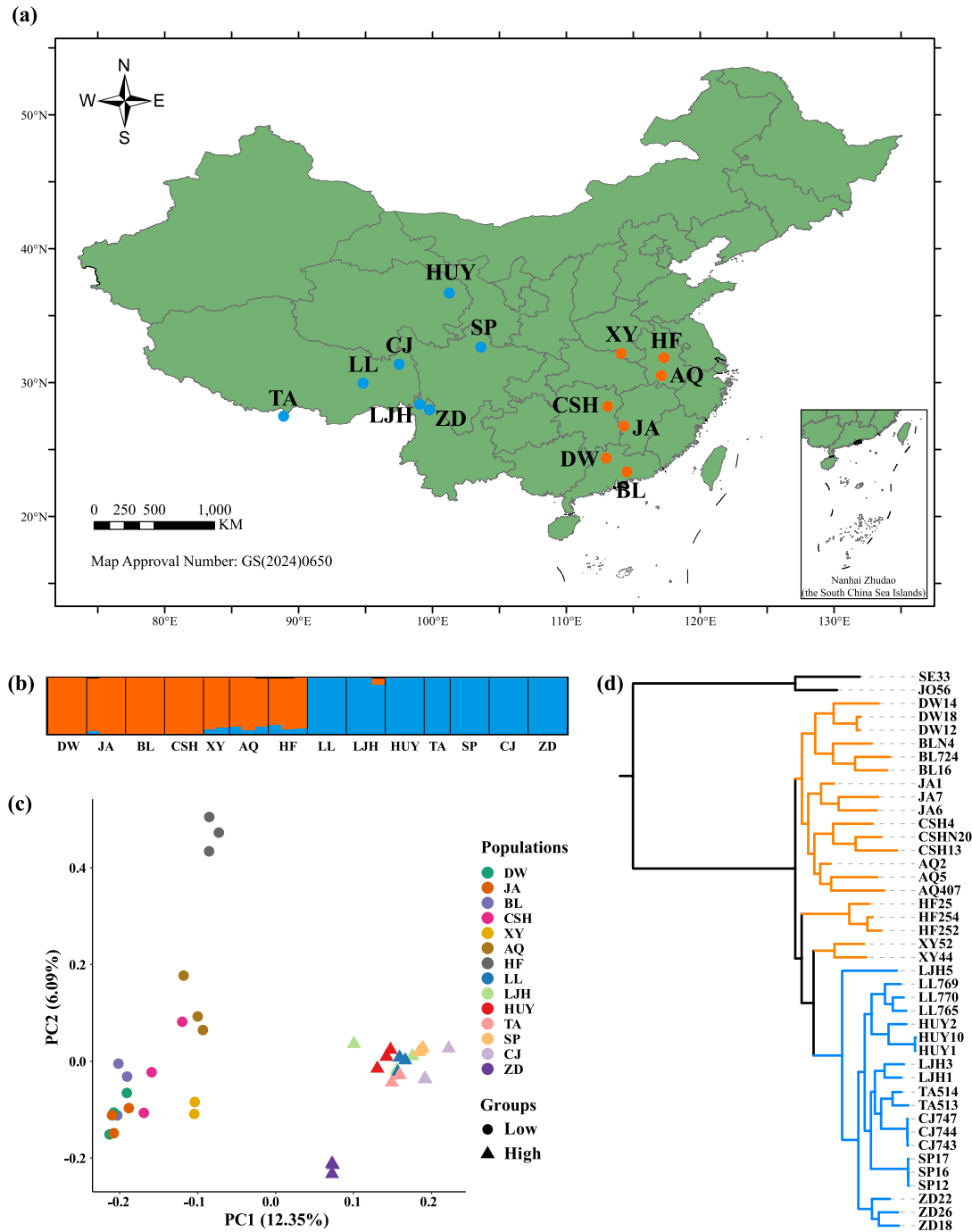


FIGURE 1 | Genetic structure of the 40 individuals from 14 populations of *Capsella bursa-pastoris*. (a) Geographic distribution of the sampled populations in China. (b) Population structure based on Admixture analysis with the best K of 2. (c) Principal component analysis (PCA). (d) Phylogenetic relationships (maximum-likelihood tree) with 100% bootstrap support for most nodes, except for three nodes with bootstrap support above 80%. [Correction added on 24 July 2026, after first online publication: Figure 1 has been updated to a revised version to ensure compliance with country-specific map regulations.]

lineage and occupy habitats with intermediate environmental conditions (Supporting Information S2: Table S2), consistent with admixture in transitional zones. In addition, individuals from LJH did not cluster tightly, consistent with admixture signals observed in the Admixture results.

3.3 | Genomic Differentiation

To detect genome-wide adaptive signatures, we first calculated pairwise F_{ST} between high- and low-altitude populations in 10-kb sliding windows. The top 1% of windows (256 in total; Figure 2a; Supporting Information S2: Table S3) were considered outliers, spanning 2.56 Mb (0.77% of the 330.91 Mb genome), indicating limited genomic differentiation. For each chromosome, the differentiated regions ranged from 0.06 to 0.25 Mb, showing localized divergence. Both CbpA and CbpB subgenomes contained an equal number of differentiated regions. The longest differentiated segments were found on CbpB, including a region on CbpB_Chr5 from 13.60 to 13.66 Mb. Based on XPCLR scores, 247 genomic regions showing signatures of selective sweeps were identified, spanning 2.47 Mb, which corresponded to approximately 0.75% of the genome (Figure 2b; Supporting Information S2: Table S4). Across individual chromosomes, the total length of selective sweep regions ranged from 0.10 Mb to 0.22 Mb, indicating limited variation in selection density and a relatively uniform genomic distribution. Notably, subgenome CbpB harbored more candidate regions under selection than subgenome CbpA (1.32 Mb vs. 1.15 Mb; 53.4% vs. 46.6%). In particular, both of the longest segments were located on CbpB, including regions on CbpB_Chr5 (13.60–13.66 Mb) and CbpB_Chr6 (0.45–0.50 Mb).

Across both the F_{ST} and XP-CLR analyses, we identified 32 genomic regions that were simultaneously defined as outliers

and thus represented strong candidates for divergent selection between high- and low-altitude populations (Supporting Information S2: Table S5). The mean F_{ST} values of these differentiated regions ranged from 0.34 to 0.82, while the XPCLR scores varied between 5.11 and 20.00. The sizes of differentiated regions per chromosome ranged from 10 to 60 kb. Outlier regions were distributed across 12 of the 16 chromosomes of *C. bursa-pastoris*, being absent only on CbpA_Chr5, CbpB_Chr1, CbpB_Chr2, and CbpB_Chr8. Notably, the CbpB subgenome harbored more candidate regions than CbpA, with 20 regions identified in CbpB compared to 12 in CbpA. This imbalance suggests that CbpB may be more strongly associated with divergence between the two lineages.

3.4 | Functional Enrichment

The outlier regions collectively contained 78 genes, with the longest differentiated segment located on CbpB_Chr5, which alone accounted for 12.82% of the total (10 genes; Supporting Information S2: Table S5). Among these genes, 54 showed an average F_{ST} value greater than 0.25 and were identified as candidate genes (Supporting Information S2: Table S6). The KEGG pathway enrichment analysis revealed that these genes were significantly enriched in metabolism-related pathways (Supporting Information S2: Table S7; Figure 3a). Among them, several pathways were significantly enriched after multiple testing correction ($FDR < 0.05$), including ATP hydrolysis-coupled proton transport, terpenoid backbone biosynthesis, pyrimidine metabolism, purine metabolism, and metabolism of terpenoids and polyketides. The genes enriched in metabolism-related pathways were grouped into four major functional categories based on their annotated functions. The first group was associated with energy and primary metabolism, including glyceraldehyde-3-phosphate

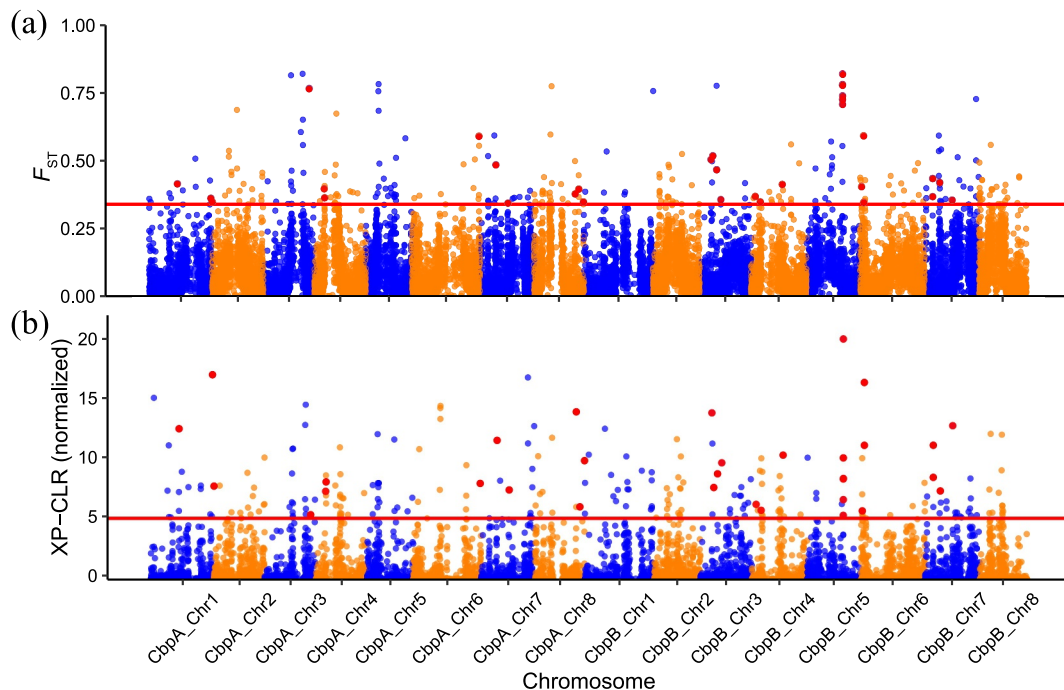


FIGURE 2 | Genomic regions under selective sweeps in *Capsella bursa-pastoris*. (a) Genome-wide distribution of F_{ST} . (b) Genome-wide distribution of normalized XP-CLR scores. Horizontal lines indicate the cutoff thresholds for F_{ST} and normalized XP-CLR scores (99% quantile). Red dots denote candidate regions under selection identified by both F_{ST} and XP-CLR analyses.

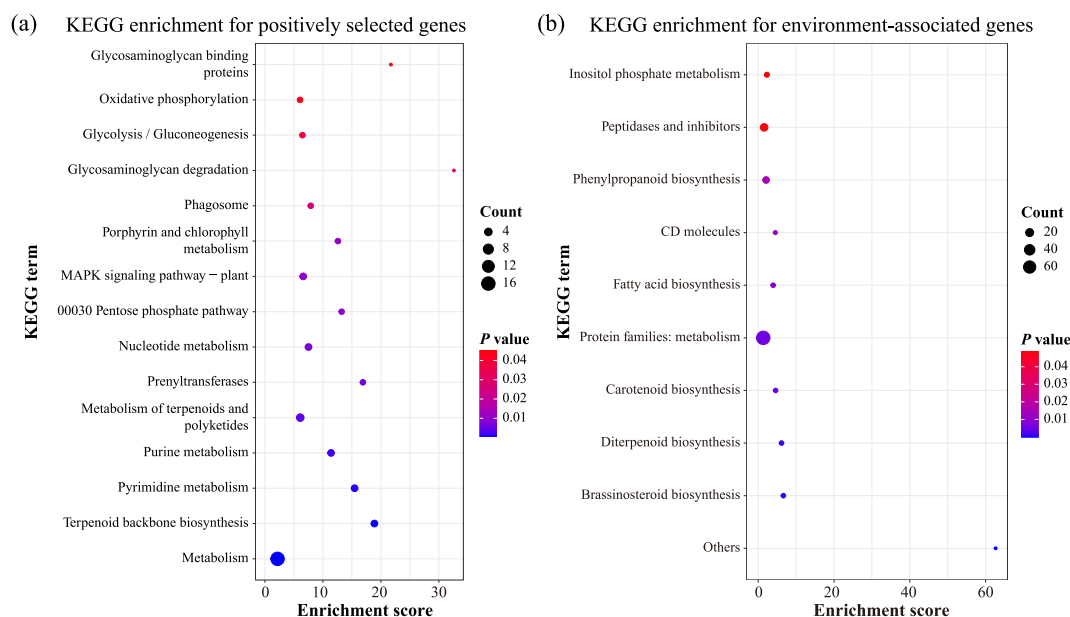


FIGURE 3 | KEGG enrichment analysis for (a) positively selected genes and (b) environment-associated genes.

dehydrogenase (Cbp_038758), members of the enoyl-CoA hydratase isomerase family (Cbp_049292), GHMP kinase family proteins (Cbp_002925), and UPP synthase family proteins (Cbp_026506). These genes are mainly involved in carbohydrate metabolism, fatty acid metabolism, and nucleotide biosynthesis, suggesting their potential role in maintaining fundamental metabolic processes. The second group was related to photosynthesis and pigment metabolism, represented by pheophorbide a oxygenase (Cbp_042720), a key enzyme involved in chlorophyll degradation. The third group comprised genes involved in signal transduction and growth regulation, such as nucleoside diphosphate kinase (Cbp_022272), adenylate isopentenyltransferase (Cbp_050025), and Pleckstrin homology domain proteins (Cbp_022273). These genes are closely related to cellular signaling, nucleotide balance, and hormone biosynthesis, pointing to their role in regulating plant development and stress responses. Finally, the fourth group included genes associated with the membrane system and molecular modification/transport, such as ATP hydrolysis-coupled proton transporters (Cbp_010242), CAAX prenyl protease 1 homolog (Cbp_020543), lecithin: cholesterol acyltransferase (Cbp_042717), and heparanase-like proteins (Cbp_044943). These genes are implicated in membrane lipid metabolism, protein post-translational modification, and extracellular matrix remodeling.

3.5 | Redundancy Analysis

To evaluate the relative contributions of climate, geographic distribution and genetic structure to population genetic variation in *C. bursa-pastoris*, we performed RDA and pRDA analysis. The full RDA model ($F \sim \text{env.} + \text{struc.} + \text{geog.}$) showed that the three factors collectively accounted for 30.7% of the total genetic variation ($p < 0.01$, Table 1). pRDA further revealed that climate alone ($F \sim \text{env.} | (\text{geog.} + \text{struc.})$) accounted for 12.7% ($p < 0.01$) of the variation, whereas geography ($F \sim \text{geog.} | (\text{env.} + \text{struc.})$) and genetic structure ($F \sim \text{struc.} | (\text{geog.} + \text{env.})$) explained only 2.3% ($p < 0.01$) and 3.9% ($p < 0.01$), respectively. These values correspond to 41.4%, 7.4%, and 12.7% of the variance explained by

the full model. Thus, when considered individually, climate had the strongest effect on genetic variation, while the effects of geography and genetic structure were relatively minor. However, when interactions among the three factors were taken into account, their combined influence on genetic variation became much more pronounced.

3.6 | Genotype–Environment Associations

Based on the pRDA model with climate variables as explanatory factors, genetic variation as the response variable, and geographic location and population structure as covariates, we performed a genome-wide scan to identify candidate loci associated with environmental variables. This approach allows environmental associations to be detected after removing the effects of geographic distance and population genetic structure. After filtering, 11 non-redundant climatic variables (bio3, bio4, bio7, bio8, bio9, bio10, bio11, bio12, bio14, bio15, and bio18) were retained for analysis (Figure 4a; Supporting Information S2: Table S8). The first two constrained axes of the RDA explained 14.4% and 6.2% of the genetic variance, respectively (Figure 4b). RDA1 was primarily associated with bio7, bio8, and bio12, while RDA2 was mainly related to bio9 and bio15 (Figure 4b). Using a significance threshold of adjusted $P/N < (0.01/1631247)$, we identified a total of 4514 environment-associated SNPs distributed across all chromosomes (102–684 per chromosome), with 2373 SNPs located in subgenome CbpA and 2141 SNPs in subgenome CbpB (Figure 4c). Notably, CbpB_Chr5 harbored the largest number of associated SNPs (684), consistent with the results from selective sweep analyses.

Among the identified SNPs, 871 were located within gene regions (spanning 638 genes), while the remaining 3643 were intergenic. GO enrichment analysis revealed that these genes were predominantly involved in vesicle trafficking and endosomal organization (i.e., receptor-mediated endocytosis, endosome transport), light and directional growth responses (i.e.,

TABLE 1 | The influence of climate, geographic location and genetic structure on genetic variation in *Capsella bursa-pastoris*.

RDA models	Variance	R^2	Proportion of total variance (adjusted R^2)	Proportion of explainable variance	p (> F)
Combined fractions					
F~env.	159,533	0.457	0.244	0.795	0.001
F~geog.	60,169	0.172	0.128	0.416	0.001
F~struc.	71,654	0.205	0.162	0.529	0.001
Individual fractions					
F~env.l(geog. + struc.)	10,8096	0.310	0.127	0.414	0.001
F~geog.l(env. + struc.)	17,682	0.051	0.023	0.074	0.002
F~struc.l(env. + geog.)	21,463	0.061	0.039	0.127	0.001
F~env.+geog. + struc.	200,143	0.573	0.307	1	0.001
Total confounded	52,902	0.152	0.118	0.385	
Total unexplained	1,431,104	0.427	0.693		
Total	1,631,247	1.000			

Note: Env., geog. and struc. represent climatic factors, geographic location factors, and genetic structure, respectively.

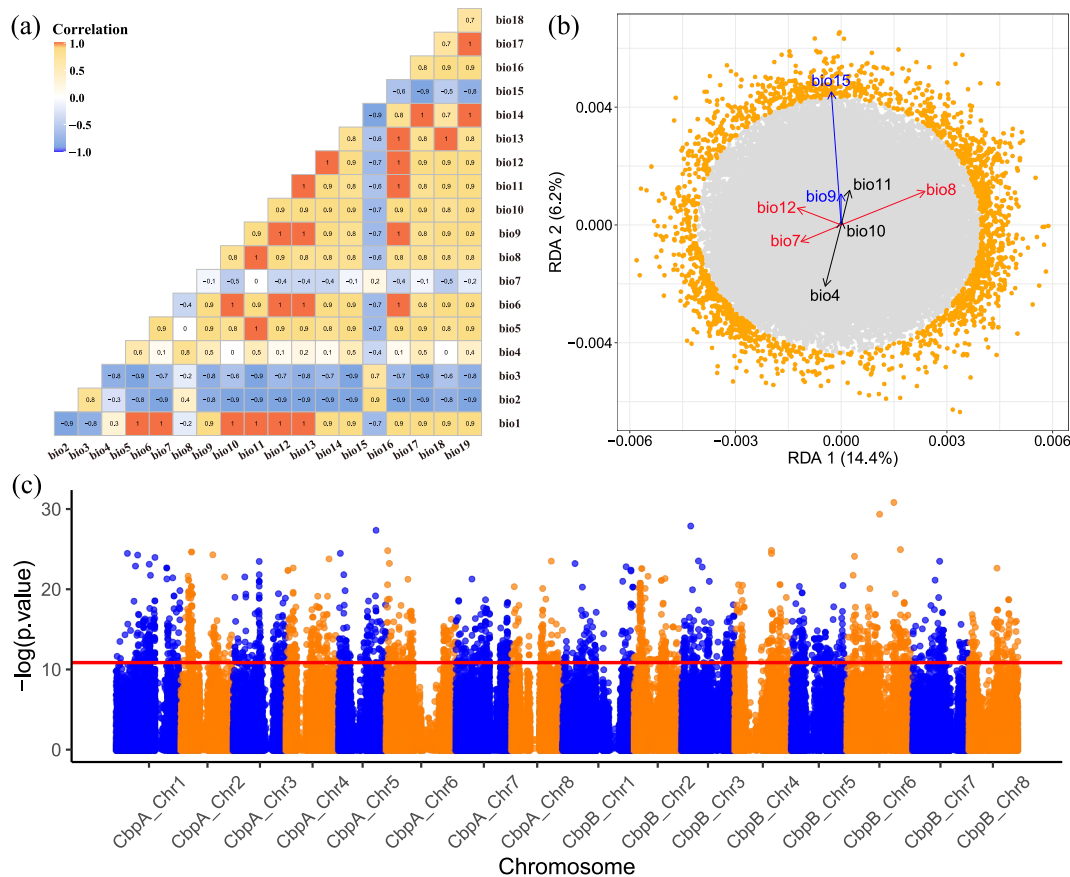


FIGURE 4 | Adaptive genetic variation in *Capsella bursa-pastoris*. (a) Correlation coefficient between each pair of the 19 environmental variables. (b) Redundancy analysis plots showing associations between adaptive loci and climatic variables. (c) Manhattan plots of SNPs associated with environmental variables, with outlier SNPs indicated above the red threshold line.

phototropism, negative gravitropism, blue light response), and developmental regulation (i.e., amyloplast organization, embryonic axis specification, embryonic pattern specification), with all enriched GO terms remaining significant after

FDR correction (corrected p -value < 0.05) (Supporting Information S2: Table S9). In addition, KEGG pathways related to metabolism showed enrichment trends (Figure 3b; Supporting Information S2: Table S10), although none reached

statistical significance after FDR correction. These results indicate that the candidate genes are associated with multiple biological processes that may underlie responses to heterogeneous climatic conditions across populations.

4 | Discussion

4.1 | Subgenome CbpB Shows an Enrichment of Differentiation Signals

This study investigated the genetic variation and divergence of *C. bursa-pastoris* based on seven high-altitude populations and seven low-altitude populations across China, with each population represented by 2–3 accessions. Although the number of individuals per population was relatively small, the sampling covers 14 populations across major geographic and environmental gradients in China, including strong contrasts in altitude, temperature, and precipitation. The genome-wide nucleotide diversity ($\pi = 0.0029$) observed in Chinese *C. bursa-pastoris* was relatively low, consistent with its predominantly selfing mating system and recent demographic expansion [13, 16, 17]. Therefore, this sampling strategy remains informative for capturing the main pattern of genetic differentiation between high- and low-altitude groups, although including more individuals could modestly improve the resolution of fine-scale variation. Our results confirmed substantial genetic differentiation between the lineages distributed in these two regions [42]. Notably, although XY and HF populations are geographically located in lowland regions, they clustered with the highland lineage. These populations are not geographically intermediate between the two lineages; instead, climatic data indicate that they occupy ecologically transitional environments. This pattern suggests that their genetic relationships are more likely explained by historical gene flow across environmental transition zones rather than by sampling artefacts or purely spatial proximity.

C. bursa-pastoris is an allotetraploid species comprising two subgenomes: CbpA, derived from the *C. grandiflora*/*C. rubella* lineage, and CbpB, derived from *C. orientalis*. Substantial divergence exists between the two subgenomes [16, 21]. In our study, CbpB shows a higher degree of genomic differentiation than CbpA. This pattern may reflect asymmetric evolutionary dynamics between the two subgenomes. CbpB originated from the selfing progenitor *C. orientalis*, which harbored lower genetic diversity and accumulated more deleterious mutations [15, 16]. However, diversity levels were similar between the two subgenomes CbpA and CbpB in this study, and a previous study even reported higher nucleotide diversity in CbpB compared with CbpA in Asian *C. bursa-pastoris* [16]. Therefore, the observed enrichment of differentiation signals in CbpB is unlikely to be solely driven by reduced background diversity or a smaller effective population size. Instead, it may reflect a combination of ancestral background, demographic history, and potential adaptive processes. Transcriptomic studies further suggest functional differentiation between the subgenomes. Although overall expression levels across China do not differ markedly between subgenomes [42], tissue-specific biases were observed, with floral tissues tending to rely more on CbpB and vegetative tissues on CbpA [43]. Taken together, these findings suggest that while both subgenomes contribute to the evolutionary trajectory

of Chinese *C. bursa-pastoris*, CbpB shows an enrichment of differentiation signals that may reflect its unique evolutionary dynamics. Moreover, the longest differentiated segment was located on CbpB_Chr5. Large differentiated blocks can result from reduced recombination (e.g., structural variation such as inversions) or strong local adaptation maintaining divergence. The presence of this region on CbpB_Chr5 suggests that it may harbor loci under particularly intense or sustained selective pressures. Further investigation of gene content and potential structural variation in this region will be needed to clarify its evolutionary role.

4.2 | Ecological Selection Drives the Divergence of Two Lineages

In China, *C. bursa-pastoris* is distributed across diverse habitats, ranging from subtropical regions in the south to cold temperate areas in the north, and from coastal lowlands to the high-altitude plateaus of the west. The strong differentiation between high- and low-altitude lineages offers valuable insights into the role of ecological selection in shaping population divergence and isolation. Although these two lineages currently occupy distinct ecological niches, the admixture populations indicate that gene flow may have occurred during their divergence. Previous studies suggest that *C. bursa-pastoris* colonized China around 1000 years ago, likely facilitated by human-mediated dispersal [17]. Following its colonization, populations likely expanded across heterogeneous habitats, during which gene flow occurred. The strong genetic structure observed between high- and low-altitude groups in our study suggests that the contrasting selective pressures across altitudinal gradients subsequently drove divergence along distinct evolutionary trajectories.

However, altitude and geography are partially confounded in our sampling design, as high-altitude populations occur mainly in the west and low-altitude populations in the east. Under such circumstances, patterns of genetic differentiation could potentially arise from isolation-by-distance (IBD) or broader spatial structure rather than ecological selection alone. To account for this possibility, we used partial redundancy analysis (pRDA) to partition the contributions of climate, geography, and population structure. When controlling for geographic distance and population structure, climatic factors alone explained 12.7% of the total genetic variation. This contribution was substantially higher than the contribution of geography (2.3%) or neutral genetic structure (3.9%), indicating that environmental gradients contribute importantly to the observed genomic divergence, beyond the effects of spatial separation alone. Building on this, our pRDA results further underscore the predominant role of ecological selection in shaping the genetic divergence of the two lineages. Distinct adaptive features are likely to have evolved in response to contrasting selective regimes. High-altitude populations experience harsher conditions, including stronger ultraviolet radiation, greater diurnal temperature fluctuations, and reduced precipitation, whereas low-altitude populations inhabit comparatively milder environments. Consistent with this, our genotype–environment association analyses revealed strong correlations between genetic variation and bio7, bio8, bio9, bio12, and bio15. These variables primarily reflect

temperature seasonality, precipitation patterns, and related climatic factors. This suggests that adaptive responses to temperature and water-use efficiency are important to the divergence between the two lineages.

Nonetheless, given the inherent spatial structure of environmental variables, some degree of shared variance between climate and geography cannot be completely ruled out. Moreover, the historical bottleneck may have strengthened genetic drift [16], further promoting divergence between the high- and low-altitude groups, whereas gene flow in several populations may have partially counteracted this drift and limited complete genetic isolation.

4.3 | Adaptive Evolution to High Altitude

Consistent with the role of ecological adaptation, we identified numerous positively selected genes (54 genes) differentiating the two lineages, providing insights into the potential mechanisms underlying the adaptation of *C. bursa-pastoris* to contrasting altitudinal environments. Several candidate genes are involved in energy and primary metabolism, processes that may help maintain cellular activity under conditions of low temperature and hypoxia [44], which are common in high-altitude habitats. Adjustments in carbohydrate, lipid, and nucleotide metabolism may ensure sufficient energy supply to sustain rapid growth and reproduction, traits characteristic of this short-lived annual weed. We also detected genes associated with photosynthesis and pigment metabolism, including pheophorbide a oxygenase, a key enzyme involved in chlorophyll degradation. Regulation of pigment turnover may help mitigate photooxidative stress under high light and ultraviolet exposure, potentially facilitating acclimation to environments with variable radiation intensity [45]. In addition, several genes are related to signal transduction and growth regulation, such as nucleoside diphosphate kinase and adenylate isopentenyltransferase, which may enable flexible adjustment of growth and developmental timing. Cytokinin biosynthesis, for instance, has been implicated in developmental regulation under environmental stress, which could be advantageous for rapid life-cycle completion in stressful environments [46]. Finally, genes associated with the membrane system and molecular modification/transport may contribute to maintaining cellular stability under fluctuating environmental conditions. Membrane-related proteins and enzymes involved in post-translational modification may contribute to maintaining membrane integrity and cellular homeostasis during environmental stress, even though the specific roles of certain proteins (e.g., heparanase-like proteins) remain to be clarified in plants [47, 48]. Collectively, these results suggest that altitudinal adaptation in *C. bursa-pastoris* may involve a combination of enhanced metabolic flexibility, photoprotection through pigment turnover, rapid growth regulation, and stabilization of membrane functions, all of which are consistent with the ecological strategy of a fast-cycling, widely distributed weed. However, the adaptive roles of these genes remain putative and will require further functional validation. In addition, given the demographic bottlenecks reported in Asian populations of *C. bursa-pastoris*, demographic history may also influence outlier detection, as strong genetic drift during bottlenecks can generate allele frequency differentiation that mimics signals of selection. Therefore, some of the detected

signals could partly reflect historical demographic processes rather than selection alone.

5 | Conclusions

In summary, this study reveals pronounced genomic divergence between the high- and low-altitude lineages of *C. bursa-pastoris* distributed across China and identifies the genetic basis underlying their local adaptation. Such environmentally associated divergence highlights the evolutionary significance of ecological adaptation in shaping population structure. Nevertheless, the observed patterns likely reflect the combined influence of ecological pressures together with background evolutionary processes, even if the latter were not the primary focus of this study. More broadly, understanding how ecological pressures shape lineage-specific adaptation provides valuable insights into the evolutionary potential of *C. bursa-pastoris*. In the face of ongoing climate change, the distinct adaptive trajectories of high- and low-altitude lineages may either enhance their persistence across heterogeneous environments or increase their vulnerability if adaptive thresholds are exceeded.

Author Contributions

Hui-Run Huang and Xue-Jun Ge designed the study. Hui-Run Huang collected samples. Lu Liu, Jia Li and Xin Liu analyzed data. Lu Liu, Hui-Run Huang and Xue-Jun Ge drafted and revised the manuscript. All authors read and approved the manuscript.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All the raw sequencing data were submitted to the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) and the Genome Sequence Archive (GSA) of the China National Center for Bioinformation (CNCB) (BioProject IDs: PRJNA1337124 and PRJCA047565).

References

1. T. J. Kawecki and D. Ebert, "Conceptual Issues in Local Adaptation," *Ecology Letters* 7, no. 12 (2004): 1225–1241, <https://doi.org/10.1111/j.1461-0248.2004.00684.x>.
2. F. Blanquart, O. Kaltz, S. L. Nuismer, and S. Gandon, "A Practical Guide to Measuring Local Adaptation," *Ecology Letters* 16, no. 9 (2013): 1195–1205, <https://doi.org/10.1111/ele.12150>.

3. O. Savolainen, M. Lascoux, and J. Merilä, "Ecological Genomics of Local Adaptation," *Nature Reviews Genetics* 14, no. 11 (2013): 807–820, <https://doi.org/10.1038/nrg3522>.
4. O. Savolainen, T. Pyhäjärvi, and T. Knürr, "Gene Flow and Local Adaptation in Trees," *Annual Review of Ecology, Evolution, and Systematics* 38, no. 1 (2007): 595–619, <https://doi.org/10.1146/annurev.ecolsys.38.091206.095646>.
5. S. N. Aitken, S. Yeaman, J. A. Holliday, T. Wang, and S. Curtis-McLane, "Adaptation, Migration or Extirpation: Climate Change Outcomes for Tree Populations," *Evolutionary Applications* 1 (2008): 95–111, <https://doi.org/10.1111/j.1752-4571.2007.00013.x>.
6. F. J. Alberto, S. N. Aitken, R. Alía, et al., "Potential for Evolutionary Responses to Climate Change – Evidence From Tree Populations," *Global Change Biology* 19, no. 6 (2013): 1645–1661, <https://doi.org/10.1111/gcb.12181>.
7. J. M. Housset, S. Nadeau, N. Isabel, et al., "Tree Rings Provide a New Class of Phenotypes for Genetic Associations That Foster Insights Into Adaptation of Conifers to Climate Change," *New Phytologist* 218, no. 2 (2018): 630–645, <https://doi.org/10.1111/nph.14968>.
8. P. F. Gugger, S. T. Fitz-Gibbon, A. Albarrán-Lara, J. W. Wright, and V. L. Sork, "Landscape Genomics of *Quercus Lobata* Reveals Genes Involved in Local Climate Adaptation at Multiple Spatial Scales," *Molecular Ecology* 30, no. 2 (2021): 406–423, <https://doi.org/10.1111/mec.15731>.
9. A. Fournier-Level, M. A. Taylor, J. F. Paril, et al., "Adaptive Significance of Flowering Time Variation Across Natural Seasonal Environments in *Arabidopsis thaliana*," *New Phytologist* 234, no. 2 (2022): 719–734, <https://doi.org/10.1111/nph.17999>.
10. Y. Q. Sun, W. Zhao, C. Q. Xu, et al., "Genetic Variation Related to High Elevation Adaptation Revealed by Common Garden Experiments in *Pinus yunnanensis*," *Frontiers in Genetics* 10 (2020): 1405, <https://doi.org/10.3389/fgene.2019.01405>.
11. X. Wang, S. Liu, H. Zuo, et al., "Genomic Basis of High-Altitude Adaptation in Tibetan *Prunus* Fruit Trees," *Current Biology* 31, no. 17 (2021): 3848–3860, <https://doi.org/10.1016/j.cub.2021.06.062>.
12. Y. L. Wang, L. Li, B. R. Paudel, and J. L. Zhao, "Genomic Insights Into High-Altitude Adaptation: A Comparative Analysis of *Roscoea alpina* and *R. purpurea* in the Himalayas," *International Journal of Molecular Sciences* 25, no. 4 (2024): 2265, <https://doi.org/10.3390/ijms25042265>.
13. H. Hurka and B. Neuffer, "Evolutionary Processes in the Genus *Capsella* (Brassicaceae)," *Plant Systematics and Evolution* 206, no. 1–4 (1997): 295–316, <https://doi.org/10.1007/bf00987954>.
14. H. Hurka, N. Friesen, D. A. German, A. Franzke, and B. Neuffer, "'Missing Link' Species *Capsella orientalis* and *Capsella thracica* Elucidate Evolution of Model Plant Genus *Capsella* (Brassicaceae)," *Molecular Ecology* 21, no. 5 (2012): 1223–1238, <https://doi.org/10.1111/j.1365-294x.2012.05460.x>.
15. G. M. Douglas, G. Gos, K. A. Steige, et al., "Hybrid Origins and the Earliest Stages of Diploidization in the Highly Successful Recent Polyploid *Capsella bursa-pastoris*," *Proceedings of the National Academy of Sciences of the United States of America* 112, no. 9 (2015): 2806–2811, <https://doi.org/10.1073/pnas.1412277112>.
16. D. Kryvokhyzha, A. Salcedo, M. C. Eriksson, et al., "Parental Legacy, Demography, and Admixture Influenced the Evolution of the Two Subgenomes of the Tetraploid *Capsella bursa-pastoris* (Brassicaceae)," *PLoS Genetics* 15, no. 2 (2019): e1007949, <https://doi.org/10.1371/journal.pgen.1007949>.
17. A. Cornille, A. Salcedo, D. Kryvokhyzha, et al., "Genomic Signature of Successful Colonization of Eurasia by the Allopolyploid Shepherd's Purse (*Capsella bursa-pastoris*)," *Molecular Ecology* 25, no. 2 (2016): 616–629, <https://doi.org/10.1111/mec.13491>.
18. A. Cornille, M. Tiret, A. Salcedo, et al., "The Relative Role of Plasticity and Demographic History in *Capsella bursa-pastoris*: A Common Garden Experiment in Asia and Europe," *AoB Plants* 14, no. 3 (2022): plac011, <https://doi.org/10.1093/aobpla/plac011>.
19. A. P. Hendry, "Key Questions on the Role of Phenotypic Plasticity in Eco-Evolutionary Dynamics," *Journal of Heredity* 107, no. 1 (2016): 25–41, <https://doi.org/10.1093/jhered/esv060>.
20. M. Orsucci, P. Milesi, J. Hansen, J. Gironde, S. Glémin, and M. Lascoux, "Shift in Ecological Strategy Helps Marginal Populations of Shepherd's Purse (*Capsella bursa-pastoris*) to Overcome a High Genetic Load," *Proceedings of the Royal Society B* 287, no. 1927 (2020): 20200463, <https://doi.org/10.1098/rspb.2020.0463>.
21. X. Liu, L. Liu, H. Yan, X. Ge, and H. Huang, "Telomere-to-Telomere Reference Genome Reveals Subgenome Divergence and Large Structural Variants in *Capsella bursa-pastoris* (Brassicaceae)," *Biological Diversity* 2, no. 1 (2025): 28–38, <https://doi.org/10.1002/bod2.70001>.
22. T. Slotte, H. R. Huang, K. Holm, et al., "Splicing Variation at a FLOWERING LOCUS C Homeolog Is Associated With Flowering Time Variation in the Tetraploid *Capsella bursa-pastoris*," *Genetics* 183, no. 1 (2009): 337–345, <https://doi.org/10.1534/genetics.109.103705>.
23. J. J. Doyle and J. L. Doyle, "A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue," *Phytochemical Bulletin* 19 (1987): 11–15.
24. A. M. Bolger, M. Lohse, and B. Usadel, "Trimmomatic: A Flexible Trimmer for Illumina Sequence Data," *Bioinformatics* 30, no. 15 (2014): 2114–2120, <https://doi.org/10.1093/bioinformatics/btu170>.
25. H. Li, "Aligning Sequence Reads, Clone Sequences and Assembly Contigs With BWA-MEM," *arXiv:1303.3997* (2013), <https://doi.org/10.48550/arXiv.1303.3997>.
26. G. A. Van der Auwera, M. O. Carneiro, C. Hartl, et al., "From FastQ Data to High-Confidence Variant Calls: The Genome Analysis Toolkit Best Practices Pipeline," *Current Protocols in Bioinformatics* 43, no. 1 (2013): 10–11, <https://doi.org/10.1002/0471250953.bi1110s43>.
27. C. C. Chang, C. C. Chow, L. C. Tellier, S. Vattikuti, S. M. Purcell, and J. J. Lee, "Second-Generation PLINK: Rising to the Challenge of Larger and Richer Datasets," *GigaScience* 4, no. 1 (2015): s13742, <https://doi.org/10.1186/s13742-015-0047-8>.
28. X. Zhao, Y. Guo, L. Kang, et al., "Population Genomics Unravels the Holocene History of Bread Wheat and Its Relatives," *Nature Plants* 9, no. 3 (2023): 403–419, <https://doi.org/10.1038/s41477-023-01367-3>.
29. N. Liu, X. Lyu, X. Zhang, et al., "Reference Genome Sequence and Population Genomic Analysis of Peas Provide Insights Into the Genetic Basis of Mendelian and Other Agronomic Traits," *Nature Genetics* 56, no. 9 (2024): 1964–1974, <https://doi.org/10.1038/s41588-024-01867-8>.
30. M. Guo, Q. Lian, Y. Mei, et al., "Analyzes of Pan-Genome and Resequencing Atlas Unveil the Genetic Basis of Jujube Domestication," *Nature Communications* 15, no. 1 (2024): 9320, <https://doi.org/10.1038/s41467-024-53718-z>.
31. P. Cingolani, A. Platts, L. L. Wang, et al., "A Program for Annotating and Predicting the Effects of Single Nucleotide Polymorphisms, SnpEff," *Fly* 6, no. 2 (2012): 80–92, <https://doi.org/10.4161/fly.19695>.
32. P. Danecek, A. Auton, G. Abecasis, et al., "The Variant Call Format and VCFtools," *Bioinformatics* 27, no. 15 (2011): 2156–2158, <https://doi.org/10.1093/bioinformatics/btr330>.
33. S. M. Musmann, M. R. Douglas, T. K. Chafin, and M. E. Douglas, "AdmixPipe: Population Analyses in Admixture for Non-Model Organisms," *BMC Bioinformatics* 21, no. 1 (2020): 337, <https://doi.org/10.1186/s12859-020-03701-4>.

34. D. H. Alexander and K. Lange, "Enhancements to the ADMIXTURE Algorithm for Individual Ancestry Estimation," *BMC Bioinformatics* 12, no. 1 (2011): 246, <https://doi.org/10.1186/1471-2105-12-246>.
35. X. Zheng, D. Levine, J. Shen, S. M. Gogarten, C. Laurie, and B. S. Weir, "A High-Performance Computing Toolset for Relatedness and Principal Component Analysis of SNP Data," *Bioinformatics* 28, no. 24 (2012): 3326–3328, <https://doi.org/10.1093/bioinformatics/bts606>.
36. B. Q. Minh, H. A. Schmidt, O. Chernomor, et al., "IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era," *Molecular Biology and Evolution* 37, no. 5 (2020): 1530–1534, <https://doi.org/10.1093/molbev/msaa015>.
37. S. Kalyaanamoorthy, B. Q. Minh, T. K. F. Wong, A. von Haeseler, and L. S. Jermin, "ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates," *Nature Methods* 14, no. 6 (2017): 587–589, <https://doi.org/10.1038/nmeth.4285>.
38. H. Chen, N. Patterson, and D. Reich, "Population Differentiation as a test for Selective Sweeps," *Genome Research* 20, no. 3 (2010): 393–402, <https://doi.org/10.1101/gr.100545.109>.
39. M. Kanehisa and S. Goto, "KEGG: Kyoto Encyclopedia of Genes and Genomes," *Nucleic Acids Research* 28, no. 1 (2000): 27–30, <https://doi.org/10.1093/nar/28.1.27>.
40. P. Dixon, "VEGAN, a Package of R Functions for Community Ecology," *Journal of Vegetation Science* 14, no. 6 (2003): 927–930, <https://doi.org/10.1111/j.1654-1103.2003.tb02228.x>.
41. C. Chen, Y. Wu, J. Li, et al., "TBtools-II: A "One for All, All for One" Bioinformatics Platform for Biological Big-Data Mining," *Molecular Plant* 16, no. 11 (2023): 1733–1742, <https://doi.org/10.1016/j.molp.2023.09.010>.
42. H.-R. Huang, J.-J. Liu, Y. Xu, M. Lascoux, X. J. Ge, and S. I. Wright, "Homeologue-Specific Expression Divergence in the Recently Formed Tetraploid *Capsella bursa-pastoris* (Brassicaceae)," *New Phytologist* 220, no. 2 (2018): 624–635, <https://doi.org/10.1111/nph.15299>.
43. D. Kryvokhyzha, P. Milesi, T. Duan, et al., "Towards the New Normal: Transcriptomic Convergence and Genomic Legacy of the Two Subgenomes of an Allopolyploid Weed (*Capsella bursa-pastoris*)," *PLoS Genetics* 15, no. 5 (2019): e1008131, <https://doi.org/10.1371/journal.pgen.1008131>.
44. X. Yang, C. Liu, M. Li, et al., "Integrated Transcriptomics and Metabolomics Analysis Reveals Key Regulatory Network That Response to Cold Stress in Common Bean (*Phaseolus vulgaris* L.)," *BMC Plant Biology* 23, no. 1 (2023): 85, <https://doi.org/10.1186/s12870-023-04094-1>.
45. A. Pružinská, G. Tanner, I. Anders, M. Roca, and S. Hörtensteiner, "Chlorophyll Breakdown: Pheophorbide *a* Oxygenase Is a Rieske-Type Iron–Sulfur Protein, Encoded by the *Accelerated Cell Death 1* Gene," *Proceedings of the National Academy of Sciences of the United States of America* 100, no. 25 (2003): 15259–15264, <https://doi.org/10.1073/pnas.2036571100>.
46. N. N. Hai, N. N. Chuong, N. H. C. Tu, A. Kisiala, X. L. T. Hoang, and N. P. Thao, "Role and Regulation of Cytokinins in Plant Response to Drought Stress," *Plants* 9, no. 4 (2020): 422, <https://doi.org/10.3390/plants9040422>.
47. L. Guerra-Guimarães, C. Pinheiro, I. Chaves, D. R. Barros, and C. P. Ricardo, "Protein Dynamics in the Plant Extracellular Space," *Proteomes* 4, no. 3 (2016): 22, <https://doi.org/10.3390/proteomes4030022>.
48. X. Su, L. Yao, X. Wang, Y. Zhang, G. Zhang, and X. Li, "Mechanisms for Cell Survival During Abiotic Stress: Focusing on Plasma Membrane," *Stress Biology* 5 (2025): 1, <https://doi.org/10.1007/s44154-024-00195-5>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting Information S1: bod270025-sup-0001-suppl-data.docx.

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