INTRODUCTION

The Arctic accounts for ~10% of Earth’s land surface, and the combination of high latitude and regional climate patterns make it one of the harshest terrestrial environments on the planet. Arctic plants must endure an extremely short and unpredictable growing season, with mean July temperatures ≤ 10°C and up to 24 hr of sunlight. The climatic conditions impose strong selective pressures for managing...
cellular and physiological responses to environmental stresses, including cold, drought (Lütz, 2010), and high light exposure (Caldwell et al., 2007). Little is known about the molecular basis of Arctic plant adaptations, and an Arctic model species has yet to be developed to facilitate such studies (Colella et al., 2020; Wullschleger et al., 2015). Exploring the molecular basis of Arctic plant adaptation may reveal novel mechanisms of environmental stress tolerance, potentially offering guidance to agricultural crop improvement.

Here, we present the chromosome-scale genome assembly of *Draba nivalis*, a perennial diploid with a circum-Arctic distribution. This species is ideal for studying the evolution of environmental stress tolerance in plants because it occurs in the high Arctic where extremes in temperature, light regime, and low water availability are ever-present. In a recent study of its transcriptome, we identified numerous candidate genes for Arctic adaptation illuminating its potential as an Arctic model species (Birkeland et al., 2020). *Draba nivalis* is also an emerging model for studying the genetics of incipient speciation as intraspecific crosses frequently result in highly sterile hybrids (Grundt et al., 2006; Gustafsson et al., 2014; Skrede et al., 2008). *Draba* is the largest genus in the Brassicaceae with >390 species, which mainly occur in Arctic and alpine regions (Jordon-Thaden et al., 2013). Many of these species, including *D. nivalis*, form dense and hairy cushions as protection from wind and cold, and are strongly autogamous to secure reproduction in their pollinator-poor environments (Brochmann, 1993). The Brassicaceae contains numerous species important for agriculture as well as for research in plant ecology, evolution, development, and molecular biology (Gupta, 2016). The availability of many Brassicaceae genome assemblies enabled us to conduct comparative analyses of chromosomal evolution and functional genomics, to shed light on the genomic characteristics of a plant adapted to the extreme abiotic stresses of the Arctic.

2 | MATERIALS AND METHODS

2.1 | Plant material and DNA sequencing

Seeds of *D. nivalis* accession 008-7 from Alaska (Waterfall Creek W., 63.045 latitude, -147.201 longitude; see Grundt et al., 2006 for complete locality information) and a plant from Norway (045-5; maternal parent; see Grundt et al., 2006 for complete locality information) were grown under conditions mimicking the Arctic climate (specified in Brochmann et al., 1992) for complete locality information) were grown under conditions facilitating such studies (Colella et al., 2020; Wullschleger et al., 2015). We generated an F2 mapping population by self-pollination of an F1 hybrid obtained from a cross between a *D. nivalis* plant from Norway (045-5; maternal parent; see Grundt et al., 2006 for complete locality information) and a *D. nivalis* plant from Alaska (008-7; paternal parent). Seeds were gently scarified before sowing and a total of 575 F2 individuals were grown to maturity under our phytotron conditions (see above). Genomic DNA was extracted from young leaf tissue using the Qiagen Plant Mini Kit, and 96-plex double-digest following the manufacturer’s protocol. Each of these four libraries was sequenced on a single Oxford Nanopore MinION flow cell (version R9) for approximately 48 hr. Two whole plants of *D. nivalis* accession 008-7 were flash frozen in liquid nitrogen and shipped to Dovetail Genomics (LLC, Santa Cruz, California 95060, USA) on dry ice for Chicago proximity ligation library preparation and 150 bp paired-end sequencing on the Illumina HiSeq 2500 platform.

2.2 | Genome assembly and scaffolding

The first draft assembly of *D. nivalis* accession 008-7 was produced using all (approximately 270 million 250 bp paired-end reads) unfiltered Illumina HiSeq data with the software DISCOVAR de novo (release 52488; https://software.broadinstitute.org/software/discover/blog/) using default parameter settings. This draft assembly (scaffold N50 = 30.083 Kb) was supplied to Dovetail Genomics for scaffolding with the HiRise pipeline using 150 bp Chicago reconstructed chromatin paired-end reads. The resulting scaffolded draft assembly (scaffold N50 = 2.92 Mb) was further improved by scaffolding with approximately 796 Mb of Oxford Nanopore 1D long reads passing the default quality filtration score in the Metrichor base calling pipeline. Long read scaffolding was first conducted using SSPACE-LongRead version 1.1 (Boetzer & Pirovano, 2014) using a minimum alignment identity of 90. These scaffolds were further improved with the Oxford Nanopore long reads using *links* version 1.8.6 (Warren et al., 2015) with K-mer size set to 21 and using 17 different distances between K-mer pairs (i.e., –d 1 Kb, 2 Kb, 4 Kb, 6 Kb, 7 Kb, 8 Kb, 10 Kb, 12 Kb, 14 Kb, 16 Kb, 18 Kb, 21 Kb, 25 Kb, 30 Kb, 40 Kb, 50 Kb, 60 Kb).

To compare broad patterns of synteny, the *D. nivalis* genome was aligned to the genomes of *A. alpina* (the most closely related species with an assembled genome; Guo et al., 2017) and *A. lyrata* with NUCmer version 4.0b2 (Kurtz et al., 2004) using all anchor matches regardless of their uniqueness (-maxmatch) and setting the minimum length of a cluster of matches (-c) to 100.

2.3 | Genetic map construction and final map-based scaffolding

We generated an F2 mapping population by self-pollination of an F1 hybrid obtained from a cross between a *D. nivalis* plant from Norway (045-5; maternal parent; see Grundt et al., 2006 for complete locality information) and a *D. nivalis* plant from Alaska (008-7; paternal parent). Seeds were gently scarified before sowing and a total of 575 F2 individuals were grown to maturity under our phytotron conditions (see above). Genomic DNA was extracted from young leaf tissue using the Qiagen Plant Mini Kit, and 96-plex double-digest
restriction-associated DNA (ddRAD) libraries were produced. For the ddRAD procedure, the restriction enzymes Nsil and Msel were used to digest 500 ng of genomic DNA per sample. Indexed P1 and P2 adapters with sticky ends matching the overhangs left by the restriction enzymes were added to the digested DNA. Following adapter ligation, individual indexed libraries were pooled and amplified with an eight cycle PCR. Ampure XP bead cleanup was performed to remove short fragments (i.e., less than ~200 bp), and the multiplexed libraries were visualized on an Advanced Analytical Fragment Analyser to ensure the libraries were of the correct size (i.e., 300–450 bp). See Supporting Information Methods for protocol details. The final multiplexed libraries were sequenced on six Illumina HiSeq 2500 lanes by the Norwegian Sequencing Centre.

Reads were demultiplexed using ipyrad version 0.5.15 (Eaton, 2014), and adapters and low-quality reads were removed using Cutadapt and FastQC available in the wrapper script Trim Galore! version 0.4.5 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/); bases with a Phred score less than 20 were trimmed, and reads shorter than 35 bp following trimming were discarded). A total of six \( F_2 \) individuals were removed because of low quality reads. Trimmed paired-end sequence reads were each mapped to the Dnv0087_Chicago assembly using bwa-mem version 0.7.8 (Li, 2013; Li & Durbin, 2010) with default settings, and duplicate reads were filtered using MarkDuplicates in Picard version 2.0.1 (http://broadinstitute.github.io/picard/). BAM alignment processing and SNP calling were performed with the Genome Analysis Toolkit version 4.1.13 (Danecek et al., 2011) resulting in a VCF file with a total of 166,644 SNPs prior to filtration. VCFtools version 0.1.13 was used to isolate biallelic SNPs and exclude any SNPs that mapped to regions of the Dnv0087_Chicago genome assembly annotated as repetitive elements (see below for repetitive element annotation). VCFtools was further used to filter the biallelic SNPs for sites with a minimum and maximum coverage depth of eight and 200, respectively, sites with a minimum mapping quality of 50, sites with a minor allele frequency greater than 0.0001, and sites that were called in at least 95% of the samples. These filtration steps resulted in a final VCF file containing 13,990 SNPs genotyped in 537 \( F_2 \) individuals.

Using these data, we constructed a genetic map using R/qtl version 1.42-8 (Arends et al., 2010; Broman et al., 2003) and ASMap version 1.42-8 (Taylor & Butler, 2017). Individuals with more than 5% missing genotypes and those that represented duplicate genotypes were removed. Loci that represented redundant genotypes were removed and an initial genetic map was estimated. Based on this map, 2,086 markers exhibiting significant segregation distortion (\( p\)-value < 1e-10) were removed and map distances were recalculated. The cross upon which this map is based is expected to contain biological sources of segregation distortion as hybrid progeny of these genotypes have previously been shown to exhibit both seed and pollen infertility (Grundt et al., 2006; Gustafsson et al., 2014; Skrede et al., 2008). To ensure that we had a genetic map as complete as possible upon which to scaffold the genome assembly, we reintegrated distorted markers into the genetic map using ASMap. The resulting genetic map and the first map that contained the distorted markers were imported into ASMap and markers exhibiting segregation distortion at \(-\log_{10} \( p\)-value < 6\) were then pushed back into the map based on the marker order reflected by the initial map, and map distances were estimated again without changing the marker ordering. The final genetic map contained 5,055 markers genotyped in 480 \( F_2 \) individuals (Figure S1, Table S1).

Chromonomer version 1.08 (http://catchenlab.life.illinois.edu/ chromonomer/) was used with default settings to scaffold the assembly based on the genetic map. To create the input files necessary to run Chromonomer, the ddRAD loci containing SNPs constituting the genetic map were first aligned to the scaffolded *D. nivalis* accession 008-7 draft genome assembly using bwa-mem (Li, 2013; Li & Durbin, 2010), and an AGP file was then generated for the scaffolded genome assembly using the script fatoagp.pl (https://github.com/sjckman/fastascripts/blob/master/fatoagp).

### 2.4 Identification and annotation of transposable elements

LTR-RTs were annotated following Choudhury et al. (2017) by identifying full-length LTR-RT copies based on structural features using ltrharvest 1.5 (Ellinghaus et al., 2008). After removal of nested as well as overlapping elements, candidate copies with internal regions matching plant non-LTR retrotransponson or DNA transponson consensus sequences from Repbase (www.girinst.org/repbase/; accessed in June 2018; Ellinghaus et al., 2008) were excluded. Internal coding regions and binding sites of remaining candidate full-length copies were annotated and classified into Gypsy and Copia superfamilies using ltrdigest (Steinbiss et al., 2009), using Hidden Markov models based on plant LTR-RT protein (Gag, Reverse transcriptase, Protease, RNaseH, Integrase, Chromodomian, and Envelope; downloaded from www.gydb.org; Llorens et al., 2011) and eukaryotic tRNA entries from the UCSC gtRNA database. Terminal inverted repeat transposons (TIR) were identified based on the presence of TIR sequences flanked by target site duplications, using GenomeTools, trivish (Gremme et al., 2013). After removal of sequences nested with other known transposable elements, putative full-length copies of TIR transposons were confirmed by inspecting internal coding domains of Class II transposons using Hidden Markov models. Helitron sequences were identified with HelitronScanner (Xiong et al., 2014) which searches for upstream and downstream termini of Helitrons within 200–20,000 bp of each other, in either the direct and reverse complement orientation following Dunning et al. (2019).

Copies of transposable elements identified in *D. nivalis* were classified into families by clustering sequences with at least 80% similarity (i.e., technical definition; El-Baidouri & Panaud, 2013; Wicker...
et al., 2009) using CD-HIT-EST (Li & Godzik, 2006). For LTR-RTs, clustering was based on LTR sequences. The full-length copy showing the lowest E-value to an HMM profile in each cluster was selected for the classification of family based on longest significant blastn hits of reverse-transcriptase domains flanked by 800 bp on either side against corresponding reverse-transcriptase sequences from Brassicaceae. Classified Brassicaceae families were further assigned to "tribes" following Choudhury et al. (2017). For TIR transposons, clustering was based on full-length copies for TIR transposons that were further classified into superfamilies (Harbinger, hAT, Mariner/Tc1, MuDR, EnSpm/CACTA) based on longest significant blastn hits against Viridiplantae DNA transposons extracted from Repbase. Helitrons show notoriously diverse internal regions and were classified following Yang and Bennetzen (2009), with clustering based on identity over 30 bp at the 3’ end of copies (i.e., the hairpin-forming region crucial for rolling circle replication).

Transposable elements were annotated along the genome assembly using all structurally defined and hierarchically classified copies of LTR-RTs, TIR transposons and helitrons from D. nivalis together with non-LTR-RTs (i.e., LINE and SINE from Viridiplantae in Repbase) as a reference. After removal of sequences giving significant blast hit with swissprot protein database for plants and with other transposable element sequences, this reference was used in RepeatMasker (version Open-4; http://www.repeatmasker.org) with RM-BLAST as search engine and divergence set to 20%. Resulting annotations of remnants of transposable element sequences were filtered to remove nested copies and copies with less than 80 bp.

2.5 | Transcriptome assembly and gene annotation

Four tissues were sampled from D. nivalis accession 008-7 for RNA extraction: young leaves (approximately 2–7 days following leaf blade expansion), mature floral buds (approximately 2–4 days prior to anthesis), open flowers (approximately 1–3 days post-anthesis), and root tissues (thoroughly washed of soil). Total RNA was isolated using the Thermo Fisher RNAqueous-Micro Kit following the manufacturer’s standard protocol for fresh tissues. A total of 30.6 μg (leaves), 22 μg (flower buds), 23 μg (open flowers), and 3 μg (roots) of total RNA was provided to the Norwegian Sequencing Centre for Illumina TruSeq Stranded RNA library preparation and sequencing. Each library was sequenced on 1/10th of an Illumina HiSeq 2500 lane to generate 125 bp paired-end reads. A de novo transcriptome assembly was generated for D. nivalis accession 008-7 using the RNA-seq data from all four tissues with the Trinity version 2.4.0 pipeline (Grabherr et al., 2011; Haas et al., 2013) including default read quality trimming and filtration using Trimmomatic version 0.32 (Bolger et al., 2014).

Genes were predicted in the D. nivalis genome assembly using the Maker version 2.31.9 (Holt & Yandell, 2011) pipeline. Augustus version 3.2.2 (Stanke et al., 2008) and SNAP (Release 2013-11-29; Korf, 2004) were used as ab initio gene predictors. The Trinity transcriptome assembly (see above) was used as transcript evidence, and protein sequences from the species Arabidopsis thaliana, Arabis alpina, Arabis salugineus, Eutrema salsugineum, Arabis alpina, Arabidopsis lyrata, and Arabidopsis thaliana (version TAIR10) were used as homology-based evidence. The D. nivalis repeat library (see above) was included to mask repetitive elements from annotation. The Maker annotation was first run using the D. nivalis transcriptome directly to infer gene predictions, and training files for the ab initio gene predictors were produced with these results. Maker was run iteratively three additional times using the transcriptome as evidence and providing updated training files for each run. The resulting set of predicted genes was annotated with Pfam domains (El-Gebali et al., 2018) using InterProScan version 5.4–47.0 (Jones et al., 2014), and GO terms were annotated using Blast2GO version 5.2.5 (Conesa et al., 2005) by searching against the UniProt (https://www.uniprot.org/) database for Viridiplantae. Both the D. nivalis genome assembly and the predicted gene set were also evaluated for completeness by searching against a set of 1,440 highly conserved plant genes (Embryophyta) using BUSCO version 3.0.1 (Simao et al., 2015). The genome assembly and predicted gene set were assessed for completeness by running BUSCO in both "genome" and "prot" modes, respectively.

2.6 | Comparative chromosome painting

Whole inflorescences of D. nivalis were fixed in freshly prepared ethanolic-acetic acid fixative (3:1) overnight, transferred into 70% ethanol and stored at −20°C until use. Mitotic and meiotic (pachytene and diakinesis) chromosome preparations were prepared as described by Mandáková and Lysak (2016a) on suitable slides pretreated with RNase (100 μg/ml, AppliChem) and pepsin (0.1 mg/ml, Sigma-Aldrich). Based on the known chromosome structure of A. alpina and other Arabideae species (Mandáková et al., 2020; Willing et al., 2015), representative BAC clones of A. thaliana were selected and grouped into contigs for comparative chromosome painting (CCP). A total of 5–10 BAC clones from each tested genomic region of A. alpina were used as hybridization probes on mitotic chromosome spreads in D. nivalis (Figure S2 Dnv1: genomic regions A and B; Dnv2: D and E; Dnv3: Fa and Fb; Dnv4: C, T and Jb; Dnv5: K-L, M-Na and M-Nb; Dnv6: O, V and S; Dnv7: Ua and Ub; Dnv8: R, W and X). The four most reshuffled chromosomes of A. alpina and other Arabideae species (Mandáková et al., 2020; Willing et al., 2015) were investigated in detail by hybridization of whole-chromosome paints (i.e. BAC contigs covering whole chromosomes except pericentromere) on pachytene chromosomes of D. nivalis (Figure 3; Dnv4, Dnv5, Dnv6 and Dnv7). The A. thaliana BAC clone T15P10 (AF167571) bearing 35S rRNA gene repeats was used for in situ localization of nucleolar organizer regions, and the clone pCT4.2 (M65137), corresponding to a 500 bp 5S rDNA repeat, was used to localize 5S rDNA loci (Figure S5). All BACs and rDNA probes were labelled with biotin-dUTP, digoxigenin-dUTP, or Cy3-dUTP by nick translation as described by Mandáková and Lysak (2016b). The labelled BACs were pooled together, ethanol precipitated, dissolved in 20 μl of hybridization mixture (50%
formamide and 10% dextran sulphate in 2× SSC) per slide and pi-
petted to a microscopic slide containing chromosome spreads. The
slide was heated at 80°C for 2 min and incubated in a moist cham-
ber at 37°C overnight. Hybridized probes were visualized either
as the direct fluorescence of Cy3-dUTP or through fluorescently
labelled antibodies against biotin-dUTP and digoxigenin-dUTP fol-
lowing Mandáková and Lysak (2016b). Chromosomes were counter-
stained with 4.6-diamidino-2-phenylindole (DAPI, 2 μg/ml) in
Vectashield antifade. Fluorescent signals were analysed and pho-
tographed using a Zeiss Axiomager epifluorescence microscope
and a CoolCube camera (MetaSystems). Individual images were
merged and processed using Photoshop CS6 software (Adobe
Systems).

2.7 | Analyses of gene family evolution

To compare the *D. nivalis* genome assembly with other Brassicaceae
species whose genomes have been sequenced, whole genome as-
semblies and associated gene annotations were downloaded from
public databases (Table S2) for the following nine species (repre-
senting three Brassicaceae clades, Guo et al., 2017): *Arabis alpina*
(clade B), *Arabidopsis lyrata* (clade A), *Arabidopsis thaliana* (clade A),
*Capsella rubella* (clade A), *Raphanus raphanistrum* (clade B), *Brassica
oleracea* (clade B), *Thellungiella parvula* (clade B), *Thlaspi arvense*
(clade B), and *Aethionema arabicum* (clade F). Among these spe-
cies, *A. alpina* is thought to be the most closely related to *D. ni-
valis* (clade B) with an assembled genome (Guo et al., 2017). As
a first analysis of gene content, we annotated Pfam domains (El-
Gebali et al., 2018) for the predicted genes of each assembly using
InterProScan (Jones et al., 2014). Pfam domains were quantified
for each species, and domains with a Z-score above 1.96 or below
–1.96 in *D. nivalis* were considered significantly enriched or con-
tracted, respectively.

To estimate gene family composition and membership
Orthofinder version 2.2.7 (Emms & Kelly, 2015, 2019) was run
using the proteins annotated in the *D. nivalis* genome and the nine
Brassicaceae genomes. OrthoFinder was run with default settings
using MMseqs2 (Steindegger & Söding, 2017) to cluster proteins by
sequence similarity. Tests for significant contractions and expansions
of gene families (defined as "orthogroups" by OrthoFinder) were
performed with CAFE version 4.2 (Han et al., 2013). The species tree
used for the CAFE analysis was generated by OrthoFinder using STAG
(Emms & Kelly, 2018) and rooted using STRIDE (Emms & Kelly, 2017).

This species tree was transformed into an ultrametric tree using
codeml of paml version 4.9i (Yang, 1997) to test for site-wise
positive selection happening on the branch leading to *D. nivalis*.
Briefly, *D. nivalis* was defined as the foreground branch on a pre-
defined phylogeny consisting of eight Brassicaceae species (*T. par-
vula*, *B. oleracea*, *R. raphanistrum*, *A. alpina*, *C. rubella*, *A. thaliana*,
*A. lyrata*, and *D. nivalis*; Table S2), and two models were compared
with a likelihood ratio test (LRT): an alternative model that allowed
positive selection on the foreground branch, and a null model that
did not allow positive selection on the foreground branch (omega
fixed to 1). The alternative model was accepted if \( \chi^2 < .05 \) (using
\( \chi^2 \) with one degree of freedom), implying that positive selection
has acted on a subset of sites along the branch leading to *D. ni-
valis*. The test was run on orthologous gene-alignments with one
gene copy from all eight species, constructed from orthogroups
identified with OrthoFinder (orthogroups are genes descended
from a single gene in the last common ancestor of the eight spe-
cies; Emms & Kelly, 2015, 2019). Due to the low number of single-
copy orthogroups, multiple copy orthogroups were divided into
subsets based on the smallest genetic distance to each of the *D.
nivalis* gene copies. This was achieved by (a) aligning all ortho-
groups based on protein sequence using MAFFT (Katoh et al., 2005);
(b) calculating Kimura protein distances (Kimura, 1983) with the
distmat algorithm in EMBOSS version 6.6.0 (Rice et al., 2000); and (c)
extracting one gene copy from all Brassicaceae species based on
the smallest protein distance to each D. nivalis gene copy. The resulting orthogroup subsets were realigned using PRANK (Löytynoja & Goldman, 2005) in GUIDANCE version 2.02 (Sela et al., 2015) with 10 bootstraps. GUIDANCE enables identification and filtration of unreliable alignment regions and sequences, and has been shown to improve positive selection inference on simulated data when used in combination with a phylogeny aware aligner like PRANK (Jordan & Goldman, 2012; Privman et al., 2012). All alignments containing sequences scoring < 0.6 and all alignment columns scoring < 0.8 in GUIDANCE were removed from the data set. Codeml was run 3–4 times for each model with different initial parameter values, and the run with the highest likelihood score was used in the final LRT (see e.g., Wong et al., 2004). Sites with ambiguity data were removed within codeml, and the species phylogeny inferred in OrthoFinder was used in all runs.

2.9 Gene ontology enrichment tests

The positively selected gene set and the sets of expanded and contracted gene families were tested for overrepresented gene ontology (GO) terms using the Bioconductor package topGO version 2.34 (Alexa et al., 2006; Gentleman et al., 2004). We used a Fisher's exact test in combination with the "classic", "elim" and "weight" algorithms to test for GO-term overrepresentation within the three domains: Biological process (BP), molecular function

TABLE 1 Assembly statistics for the D. nivalis genome. Each row shows a different stage in the scaffolding process of the genome assembly. For a more complete table, see Table S3
The three algorithms differ in that the "classic" algorithm processes each GO-term independently without considering the GO-graph, the "elim" algorithm processes the GO-graph bottom-up while discarding genes that have already been mapped to significant GO-terms, and the "weight" algorithm is weighing genes annotated to a GO-term based on the scores of neighboring GO-terms (Alexa et al., 2006). Based on simulated data, the "weight" algorithm has been shown to produce less false positives than the "classic" algorithm, whereas the "elim" algorithm further reduces false-positive rate, but with a higher risk of discarding true positives (Privman et al., 2012). The *D. nivalis* annotated gene set was used as a custom background for all GO term enrichment tests. The significance level was set to $p < .05$, and the results were not corrected for multiple testing following the recommendations of the creators of the topGO package (Gentleman et al., 2004).

### RESULTS

#### 3.1 | Genome assembly

Based on the 25mer frequency distribution we estimated the genome size of *D. nivalis* to 278.48 Mb (Figure S4; flow cytometry estimates report 254–308 Mb; Grundt et al., 2005). The initial de novo draft assembly (based on the Illumina paired-end HiSeq data) had a scaffold N50 of 30.083 Kb and a length of 280.94 Mb. Scaffolding this assembly with 1.57 Gb of Chicago proximity ligation data using the HiRise pipeline resulted in a scaffold N50 of 2.92 Mb and a length of 300.29 Mb. Scaffolding with 1.33 Gb of Oxford Nanopore MiniON long read data (207,896 reads ranging in length from 1 Kb to 158.14 Kb with a mean read length of 3.8 Mb) further improved the scaffold N50 to 4.44 Mb and the length to 301.71 Mb (Table 1; Table S3). We also produced a linkage map using 480 F2 individuals genotyped with 5,055 SNPs (Figure S1) to order scaffolds into eight pseudomolecules, referred to as chromosomes (see Methods). The final assembly is 301.64 Mb (scaffold N50 = 31.02 Mb), of which 276.24 Mb is anchored to chromosomes varying from 29.2 Mb to 43.1 Mb (Figure 1a).

#### 3.2 | Chromosome evolution

To examine how the *D. nivalis* genome conforms to broader patterns of genome evolution in the Brassicaceae, we compared pairwise synteny between chromosomes of *D. nivalis* and those of *Arabidopsis lyrata* and *Arabis alpina*, and performed comparative chromosome painting (CCP) experiments to identify genomic blocks of the Ancestral Crucifer Karyotype (ACK, Schranz et al., 2006, Lysak et al., 2016; represented by the *A. lyrata* genome; Figures 1b, 2, and 3; Figures S2 and S3). By synthesizing these results, we inferred the structure of the *D. nivalis* chromosomes. We identified several rearrangements and extensive centromere repositioning relative to the ACK. The structure of the *D. nivalis* genome is very similar to that of *A. alpina* (Willing et al., 2015), the closest relative of *D. nivalis* for which a chromosome-scale genome assembly is available, and consistent with genome structures determined for other Arabideae species, including three *Draba* species (Mandáková et al., 2020).

![FIGURE 2](https://example.com/Figure2.png) Syntenic relationships between *D. nivalis* and *Arabidopsis alpina* (a) and *Arabidopsis lyrata* (b). The *D. nivalis* genome was aligned to the genomes of *A. alpina* and *A. lyrata* with NUCmer. Chromosomes are colour-coded to match the Ancestral Crucifer Karyotype (ACK; Lysak et al., 2016) structurally resembling the *A. lyrata* genome (b) [Colour figure can be viewed at wileyonlinelibrary.com]
3.3 | Repetitive element annotation

We annotated 94.8 Mb of the genome as direct remnants of repetitive elements, dominated by long terminal repeat retrotransposons (LTR-RT, 60.1 Mb), terminal inverted repeat transposons (TIR, 20.9 Mb), and Helitrons (13.4 Mb; Figure 1; Tables S4–S6). Consistent with A. alpina, A. lyrata, and A. thaliana, LTR-RT density increases in pericentromeric regions of each chromosome, TIR density decreases in pericentromeric regions, and Helitron density is stable along chromosomes. Abundance of LTR-RT Copia and Gypsy elements is similar to that of A. alpina (Choudhury et al., 2017), whereas TIR-CACTA elements and Helitrons seem to be particularly abundant in D. nivalis (Hu et al., 2019). Nucleotide divergence among LTR-RTs identifies several Copia and Gypsy LTR-RTs showing recent transposition bursts across the genome of D. nivalis. Some abundant LTR-RTs (e.g., ALYCopia74, ATLANTYS2) have very similar copies (most > 98%) and thus seem to have proliferated more recently than in A. alpina (Figure 4). These results show that Copia elements, including the heat-activated ATCOPIA78 (Ito et al., 2011) and tribes preferentially transposing across the gene space of Brassicaceae (Quadrana et al., 2016), have specifically contributed to the evolution of the D. nivalis genome.

3.4 | Gene annotation

We predicted gene models with the Maker2 pipeline using BLAST homology to five Brassicaceae genomes and a de novo transcriptome assembly of D. nivalis based on RNA-seq data from leaves, roots, flowers, and flower buds (see Methods). We identified 33,557 gene models, and 74% of the genes were functionally annotated based on similarity to UniProtKB entries, and 70% were annotated with InterPro domains. Approximately 58% of the 33,557 gene models had an annotation edit distance less than or equal to 0.25, suggesting a relatively high degree of agreement between predicted gene models and external evidence. This gene set is somewhat larger than that of A. thaliana (27,654), but consistent with those of closely related species with similar genome size (Figure S5), and BUSCO analyses indicate 95.2% completeness of conserved embryophyte genes (Table S7). The average gene density in D. nivalis is approximately one gene per 9 Kb, and similar to A. thaliana, A. lyrata, and A. alpina, gene density decreases towards the centromeres (Figure 1a; Willing et al., 2015).

3.5 | Gene and gene family evolution

To explore specialization in the D. nivalis gene set, we compared the abundance of protein family (Pfam) annotations with those of nine Brassicaceae genomes representing broad phylogenetic sampling. We found 226 Pfam domains to be significantly enriched and 32 to be significantly depleted relative to the other species (Table S8). To summarize functional associations of the enriched Pfam domains, we extracted gene ontology (GO) terms from their corresponding InterPro entries. Amongst these GO terms, the most common
biological process (BP) GO term is “oxidation-reduction process”. Numerous environmental stimuli and stresses can lead to the production of reactive oxygen species (ROS), which can damage cell membranes, nucleic acids, proteins, and metabolites (Apel & Hirt, 2004). Regulation of ROS metabolism is essential for maintaining cellular oxidation-reduction (redox) homeostasis and is an integral part of the intracellular signal transduction networks evoked by external stimuli (Mittler, 2017), particularly for responses to environmental stresses induced by light, drought, and cold (Neill et al., 2002). The significant increase in Pfam annotations involved in redox processes in D. nivalis may indicate that it has evolved novel ways to cope with ROS accumulation associated with Arctic environmental stress (Figure 5). The salt tolerant Eutrema salsugineum (syn. Thellungiella salsuginea), for example, responds to salt stress by expressing an aldehyde dehydrogenase, a scavenger of toxic aldehydes produced as a byproduct of ROS accumulation (Hou & Bartels, 2014). Consistent with this, the D. nivalis annotated gene set contains 26 genes containing the significantly enriched molybdopterin-binding domain of aldehyde dehydrogenase.

To compare the diversity and abundance of D. nivalis gene families relative to the nine other Brassicaceae species, we estimated gene family (orthogroup) membership using OrthoFinder. A total of 29,194 (87%) D. nivalis genes were assignable to one of the 21,635 gene families identified, and 10,401 of the gene families contain at least one gene copy in all 10 species. Genome-wide classification of gene duplications in D. nivalis using the Dup_GenFinder pipeline (Qiao et al., 2019) resulted in similar patterns across all species (Table S9). Gene duplications in D. nivalis are dominated (22,989 gene pairs, 89.6%) by transposed (TRD, 7,308 pairs) and dispersed (DSD, 15,681 pairs) duplicates (Figure 6a), both of which are probably the product of transposition events that can be mediated by transposable elements (Qiao et al., 2019).

Relative to the nine other species, D. nivalis contains 198 significantly expanded and 31 significantly contracted gene families (Figure 6a). Exploring the functional annotations of the 2,958 genes of the expanded gene families (EGFs), we found 158 significantly enriched BP GO annotations including several functions highlighting how this species was able to adapt to Arctic habitats (Figure 6b; Tables S10–S12). Functions associated with stress signaling include both the abscisic acid (ABA) activated and brassinosteroid-mediated pathways, involved in cellular functions including abiotic stress signaling (Planas-Riverola et al., 2019).
Functional enrichment for heat acclimation associated with three EGFs, which also are associated with defense responses to fungal pathogens. While fungal pathogens are not expected to be particularly virulent in the Arctic, stress can make plants more susceptible to pathogens. The EGFs are also enriched for functions associated with meiosis, specifically the assembly of the synaptonemal complex. The efficiency and fidelity of recombination is sensitive to temperature (Bomblies et al., 2015), and these results may indicate adaptation in *D. nivalis* to facilitate meiosis in cold habitats. Gene families associated with desiccation resistance are also expanded in *D. nivalis*, consistent with its occurrence in extremely dry, so-called polar deserts. While BP terms related to redox homeostasis were not enriched in the EGFs (Figure 6b), redox activity was prominent among the 64 enriched MF terms (Figure 6c), consistent with the overrepresentation of redox Pfam domains observed in the genome (Figure 5). Functions associated with protein modification and ubiquitination were enriched in the EGFs, consistent with previously published results for the salt tolerant *Thellungiella salsuginea* (Yang et al., 2013). Finally, the *D. nivalis* genome contains several EGFs that function in histone binding and methylation, integral parts of epigenetic regulatory mechanisms that can play important roles in numerous abiotic stress signaling and response pathways (Ueda & Seki, 2020). Patterns of duplication inferred for the 2,958 genes that constitute the EGFs in *D. nivalis* are broadly consistent with genomic
patterns in that TRD and DSD duplications dominate (79.9%), but TRD duplications are more frequent, and proximal (PD) and tandem (TD) duplications are more frequent in EGFs than would be expected by chance (Figure 6a, Table S9). This suggests that the activity of prevalent LTR-RTs, TIR transposons, and Helitrons probably played important roles in the expansion of D. nivalis gene families, but processes of proximal and tandem duplication also appear to have been important in the expansion of gene families associated with protein modification, stress signalling, desiccation resistance, and defense responses to fungal pathogens (Figure 6b,c).

### 3.6 Tests of positive selection

To search for further evidence of Arctic adaptation in D. nivalis, we performed genome-wide positive selection tests to identify genes that probably evolved under positive selection in this lineage relative to seven related species (see Methods). We found 1,307 positively selected genes (PSGs). These include several candidate genes with functions directly relevant to typical environmental stresses of the Arctic, associated with “response to cold”, “response to water deprivation”, “photoperiodism”, “response to oxidative stress”, and
“meiosis I” (Figure 7a,b; Tables S13 and S14). Patterns of functional enrichment of the PSGs also highlight several significant BP GO terms probably connected to Arctic adaptation, including “vernalization response”, “drought recovery”, “short-day photoperiodism”, and “oxidation-reduction process” (Figure 7b; Table S14). We also found four PSGs associated with meiosis I, including two D. nivalis homologues to A. thaliana ZYP1A, which is one of three synaptonemal complex transverse filament proteins whose function is disrupted by temperature stress (Bomblies et al., 2015). These results provide evidence for the likely adaptive evolution of core meiosis genes reflected both in EGFs (Figure 6b) and in positive selection acting on specific components of the synaptonemal complex.

4 | DISCUSSION

Summarizing the results of our comparative genomic analyses, we observe some similarities in functional patterns among enriched Pfam domains, gene family expansions, and genes under positive selection (Figure 7c,d). Our results reveal a multifaceted landscape of stress adaptation in the D. nivalis genome, and highlight the important roles that genes involved in stress signaling/response, redox homeostasis, light sensing, and meiosis probably play in plant adaptation to the extreme Arctic environment. The numerous genes that we have identified represent good candidates for future studies of functional validation in various stress responses. If such studies are.

**FIGURE 7** Genes under positive selection in D. nivalis. (a) Distribution of the ratios of ln likelihoods (lnL) from tests for positive selection in 15,828 D. nivalis genes. Genes with a higher proportion of nonsynonymous to synonymous substitutions have a higher lnL ratio, and those with an lnL ratio above the X2 critical value (3.84, dashed line, p-value < 0.05, df=1) are considered significantly likely to contain codons that evolved under positive selection in D. nivalis (PSGs; see Methods). Coloured dots represent genes that are annotated with biological process (BP) GO terms of particular interest for Arctic adaptation. (b) Summary of key BP GO terms in the D. nivalis PSGs. Asterisks (*) indicate significantly enriched terms relative to the genomic background. Parent terms are in bold. (c and d) Venn diagrams showing the overlap of BP (c) and molecular function (d) GO terms resulting from analysis of Pfam domains, expanded gene families (EGF), and PSGs in D. nivalis (see also Table S10) [Colour figure can be viewed at wileyonlinelibrary.com]
successful, they could provide guidance for various approaches to crop improvement. The highly contiguous genome assembly of *D. nivalis* that we have produced provides numerous avenues for the continued development of this species as the first Arctic specialist model plant. Future uses of this resource could include, e.g., studies of the adaptive potential of Arctic species to future climate change.

**ACKNOWLEDGEMENTS**

We thank Filip Kolár for helpful discussions. The main work was funded by grant 240223/F20 to CB from the Research Council of Norway; additional support was obtained from the Czech Science Foundation (grant 15-18545S) and the CEITEC 2020 project (grant LQ1601). Computational analyses were performed on resources provided by UNINETT Sigma2 - the National Infrastructure for High Performance Computing and Data Storage in Norway, and on the Abel Cluster, owned by the University of Oslo and UNINETT Sigma2, and operated by the Department for Research Computing at USIT, the University of Oslo IT-department (http://www.hpc.uio.no/).

**AUTHOR CONTRIBUTIONS**


**DATA AVAILABILITY STATEMENT**

The raw data (shotgun sequence data, MinION long reads, Chicago Linked Reads, and RNA-seq) have been deposited in the NCBI SRA with Bioproject number PRJNA657155. The final chromosome-scale assembly, gene annotation, repeat library annotation, and annotation, transcriptome assembly, ddRAD sequence data of the F2 mapping population, and vcf file of variants used in the construction of the genetic map are available on Dryad (https://doi.org/10.5061/dryad.pg4f4qrm4).

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