Phylogeographic patterns of *Deschampsia cespitosa* (Poaceae) in Europe inferred from genomic data

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The highly variable tufted hairgrass *Deschampsia cespitosa* is a tussock-forming plant especially of cool and humid environments. Although common and widespread, its phylogeographic structure and the significance of polyploidy for its evolution are poorly understood. Here we used a phylogenomic approach to study the genetic structure of this species in Europe and how the polyploid cytotypes/subspecies are related to the diploids. Using genomic data (RADseq and whole plastid sequencing) we found a highly divergent Iberian group, including the Spanish *Deschampsia cespitosa* subsp. *cespitosa* (diploid and tetraploid) and the Macaronesian island endemic diploid *Deschampsia argentea*. Moreover, we found substantial divergence of pseudoviviparous tetraploids (*Deschampsia cespitosa* subspp. *neoalpina* and *rhenana*) from seminiferous tetraploids (except *Deschampsia cespitosa* subsp. *littoralis*) and all diploids of the remaining European samples. The divergent pseudoviviparous tetraploids (*D. cespitosa* subspp. *neoalpina* and *rhenana*) and the seminiferous tetraploid *D. cespitosa* subsp. *littoralis* probably represent periglacial and relict lineages of unknown origin regarding auto- and/or allo-polyploidy, whereas other seminiferous tetraploid variants of *D. cespitosa* are always nested in the diploid *D. cespitosa*, suggesting multiple autopolyploid origins. An analysis after excluding the Iberian Group and the highly divergent tetraploids revealed five genetic groups with overlapping geographical patterns. However, the recovered geographical structure, the overall low genetic divergence and the diffuse genetic structure point to recolonization from various refugial areas and secondary contact. Effective wind dispersal of pollen and seeds in an open early post-glacial tundra landscape and, finally, increasing human impact on dispersal of this grass since the Neolithic, may have enhanced admixture and resulted in the complex patterns detected today.

ADDITIONAL KEYWORDS: allopolyploidy – autopolyploidy – diploids – genomic structure – plastids – RADseq – tetraploids.

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INTRODUCTION

Phylogeographic structure of organisms is a result of both past and recent events. Pleistocene climate oscillations had a strong impact on the distribution and genetic structure of plant species. Due to range contraction, most warm-adapted species became isolated in cold periods, and migrated/expanded in warmer periods, whereas the distribution of cold-tolerant species was favoured during cold periods followed by range contraction in interglacial periods (Comes & Kadereit, 1998; Taberlet *et al.*, 1998; Hewitt, 1999; Tzedakis *et al.*, 2013; Kajtoch *et al.*, 2016; Hedrén *et al.*, 2018; Schönswetter & Schneeweiss, 2019; Šrámková *et al.*, 2019). However, from the Neolithic up to the present (Anthropocene), human activities have had an increasing impact on plant migration and establishment (e.g. Balfourier *et al.*, 2000; Nathan, 2006; Cornille *et al.*, 2012; Kueffer, 2017; Amirchakhmaghi *et al.*, 2018; Liu *et al.*, 2019).

Plant migration, i.e. dispersal of propagules, is often modelled using dispersal kernels depending essentially on propagule features, which roughly allow the probability of a propagule to be found within the space between the source and an end point to be calculated (Nathan *et al.*, 2012). The importance of generalized or occasional extreme vectors for long-distance dispersal (LDD) (Nathan, 2006; Meza Torres *et al.*, 2015), and the role of intrinsic features beyond dispersal ability favouring establishment and survival in a new area (Alsos *et al.*, 2007) have been discussed in this context. Although rare and unpredictable, LDD has emerged as the most plausible explanation to account for the existence of a specific taxon or related taxa on different continents or on remote isolated archipelagos such as Hawai'i, the Tristan da Cunha Archipelago and the Juan Fernandez Islands (Cain *et al.*, 2000; Givnish & Renner, 2004; de Queiroz, 2005; Gillespie *et al.*, 2012; Takayama *et al.*, 2015). Plants that are able to establish under various conditions and occupy a broad ecological niche may find favourable conditions on a wide range of habitats in several biomes.

Many grasses are among the plants with such potential. Linder *et al.* (2017) reviewed the role of functional traits in the global success of the grass family (Poaceae) pointing to their capacity to even transform environments (the 'Viking syndrome'). Specifically, the distribution of some common grass genera is assumed to result from LDD due to intercontinental distributions, e.g. *Arundo* L. (Hardion *et al.*, 2014), *Festuca* L. (Inda *et al.*, 2008), *Hordeum* L. (Blattner, 2006) and *Munroa* Torr. (Amarilla *et al.*, 2015). In part, on a global scale, this is true also for *Deschampsia cespitosa* (L.) P.Beauv., which is the most common species of the genus, found on all continents, disjunct among all Northern Hemisphere landmasses,

southern South America, Australasia and South Africa. It is a tussock-forming, wind-pollinated, self-incompatible grass (Chiapella, 2000).

Deschampsia cespitosa is an extremely tolerant grass thriving best under conditions of low temperatures and high moisture. The grass is found in damp areas, along the shores of lakes and streams (Davy & Taylor, 1975), from moist grasslands of lower elevations up to various mountain habitats and alpine tundra (Davy, 1980). It is a frost-hardy plant (Lawrence, 1945) that can even thrive under permafrost conditions (Tieszen & Bonde, 1967). The grass was present in the Yukon Territory 25 000 years ago (Froese *et al.*, 2006) and, given a similar or even more moderate climate, we can assume that it was also a component of the Ice Age grass flora in Europe and Asia. The findings of abundant grass and sedge remains in the stomach contents of frozen Eurasian Pleistocene herbivores (Vereshchagin & Baryshnikov, 1992) indicates a high abundance of these plants in the permafrost regions although in the Ice Age vegetation forbs may have dominated. Willerslev *et al.* (2014) identified forbs as the major megafaunal diet during the last Ice Age and a transition from a dry to a moist tundra favouring grasses in the last 10 000 years. At present *Deschampsia cespitosa* subsp. *borealis* (Trautv.) Tzvelev is found in the flood plains of the Lena River Delta in the north-eastern Siberian zone of continuous permafrost (Boike *et al.*, 2013), whereas *Deschampsia cespitosa* subsp. *neoalpina* Chiapella, Xue & Greimler occurs in northern Europe, Greenland and Arctic North America in moist glacier forelands (Whittaker, 1991) or close to water courses at higher elevations (Norway: Sandvik & Odland, 2014; Iceland: J. Greimler, pers. obs.). In the Southern Hemisphere, the closely related *Deschampsia antarctica* E.Desv. thrives on gelisols on the permafrost ground of the sub-Antarctic South Orkney Islands (Guglielmin *et al.*, 2008). In central Europe, the upper elevational limits of *D. cespitosa* are found in the alpine grasslands and glacier forelands of the Alps (Erschbamer *et al.*, 1999, 2010; Fickert *et al.*, 2016).

The high variability and the broad ecological niche of *D. cespitosa* have led to the description of many variants on several taxonomic ranks especially in the Northern Hemisphere. For Europe, we follow Chiapella (2000) and Clarke (1980) ranking all those variants as subspecies of *D. cespitosa* (Table 1). However, in our a priori classification we consider also the findings on notable genetic and ecological separation of *Deschampsia cespitosa* subsp. *littoralis* (Gaudin) Gremli (Peintinger *et al.*, 2012) and *Deschampsia cespitosa* subsp. *wibeliana* Griseb. (Heydel *et al.*, 2017) from common *Deschampsia cespitosa* (L.) P.Beauv. subsp. *cespitosa*. Among the tetraploids, *D. cespitosa* subsp. *neoalpina* was considered a polyphyletic

Table 1. Classification and distribution of *D. cespitosa* and its subspecies in Europe including the Macaronesian endemic species *D. argentea*

A priori classification in this study	Ploidy	Pseudovivipary	Included in this study	Distribution	Chiapella (2000)	Peintinger et al. (2012)	Heydel et al. (2017)
subsp. *bottnica*	2x	No	No	Baltic Sea shores	subsp. *bottnica*		
subsp. *cespitosa*	2x, 4x	No	Yes	Entirely in Europe	subsp. *cespitosa*	*Deschampsia cespitosa*	*Deschampsia cespitosa*
subsp. *glauca*	2x	No	No	Northern Scandinavia	subsp. *glauca*		
subsp. *littoralis*	4x	No	Yes	Shores of pre-Alpine lakes	Included in subsp. *rhenana*	*Deschampsia littoralis*	
subsp. *neoalpina*	4x	Yes	Yes	Northern Hemisphere mountains	subsp. *alpina*		
subsp. *parviflora*	2x	No	Yes	Central and western Europe	subsp. *parviflora*		
subsp. *rhenana*	4x	Yes	Yes	Shores of Lake Constance	subsp. *rhenana*	*Deschampsia rhenana*	
subsp. *wibeliana*	2x	No	No	Elbe Estuary (Germany)	subsp. *paludosa*		*Deschampsia wibeliana*
Deschampsia argentea	2x	No	Yes	Madeira, endemic			

assemblage of mostly clonal variants occurring in the mountains of the Northern Hemisphere (Hedberg, 1958; Kawano, 1963). Aiken *et al.* (2007) suggested that *D. cespitosa* subsp. *neoalpina* could be either an autopolyploid derived from *D. cespitosa* subsp. *cespitosa* or an allopolyploid involving Arctic *Deschampsia brevifolia* R.Br. Apart from the extreme modification of spikelets due to pseudovivipary (asexual reproduction: spikelets developed into small plantlets), the morphological differences with *D. cespitosa* subsp. *cespitosa* are few and suggest the treatment at the subspecific rank is more appropriate (Rothera & Davy, 1986; Chiapella, 2000; Chiapella & Probatova, 2003). The other pseudoviviparous and tetraploid subspecies, *Deschampsia cespitosa* subsp. *rhenana* (Gremli) Kerguélen, was shown to be genetically separated from seminiferous tetraploid *D. cespitosa* subsp. *littoralis* and diploid *D. cespitosa* subsp. *cespitosa* by Peintinger *et al.* (2012).

Phylogenomic relationship inference is now widely used owing to the rapid advances in next-generation sequencing technology. Restriction site-associated DNA sequencing (RADseq: Miller *et al.*, 2007; Etter *et al.*, 2011), which provides a reduced representation of the genome, is now widely applied to phylogeographic and systematic studies (e.g. Guo *et al.*, 2020; Hipp *et al.*, 2020). In contrast to genomic-level sequencing, gene markers only provide a limited number of polymorphic loci and low resolution. Many studies of non-model groups implemented a methodology including *de novo* assembly of selected genomic regions to resolve the genetic structure and phylogenetic relationships (e.g. Paun *et al.*, 2016; Trucchi *et al.*, 2017), which requires high coverage data, whereas reference-based analyses perform well even for low coverage data. A closely-related reference genome sequence could improve the ability to detect more SNPs and filter paralogous and repetitive sequences (e.g. Shafer *et al.*, 2016; Guo *et al.*, 2020). Guo *et al.* (2020) further compared reference-based ddRAD data assembly approaches using four published genomes in Poaceae and *de novo* assemblies in phylogenetic inference of the polyploid tribe Arundinarieae and confirmed that reference-based approaches perform better in identifying orthologues in polyploids. In *Deschampsia*, previous studies mainly used morphological traits and one to few DNA markers (nuclear or plastid DNA regions) to study phylogenetic relationships (Chiapella, 2007; Chiapella *et al.*, 2011), showing low genetic variation in this genus.

Here we investigate the biogeography of the tufted hairgrass *D. cespitosa s.s.* (i.e. *D. cespitosa* subsp. *cespitosa*) in Europe and its relation to the European polyploid variants/subspecies. The specific questions are as follows: (1) What is the genetic structure of *D. cespitosa* in Europe? (2) What are the patterns of

(re)colonization of former glaciated regions (Alps, northern Europe)? (3) What are the relationships among diploid and tetraploid seminiferous and pseudoviviparous populations?

MATERIAL AND METHODS

THE STUDY SPECIES AND PLANT MATERIAL

Deschampsia cespitosa is a perennial grass with small spikelets and mostly two florets. With few exceptions, genome size estimates and representative chromosome counts (Greimler *et al.*, 2022) showed a diploid status ($2n = 2x = 26$) in Europe which agrees with earlier chromosome counts by other authors (e.g. Kawano, 1963; Albers, 1975, 1980; Garcia-Suarez *et al.*, 1997; Dobeš & Vitek, 2000). Besides the widely distributed diploids, several seminiferous tetraploids in the United Kingdom, Italy, France, Spain, North Macedonia and Switzerland have been found that are indistinguishable from diploids by gross morphology. Pseudoviviparous plants [*D. cespitosa* subspp. *neoalpina* (the former epithet *alpina* is illegitimate according to Chiapella *et al.*, 2021) and *rhenana*] that appear morphologically different due to modifications in their spikelets were always found to be tetraploid (Greimler *et al.*, 2022). Here we have included *D. cespitosa* subspp. *cespitosa*, *neoalpina*, *littoralis*, *rhenana* and *Deschampsia cespitosa* subsp. *parviflora* (Thuill.) K.Richt. Additionally, we included *Deschampsia argentea* Lowe, endemic to Macaronesia, because of its high morphological affinity (broad leaves) to *D. cespitosa*, although Buschmann (1950) considered it to be derived from *Deschampsia setacea* (Huds.) Hack. or *Deschampsia media* (Gouan) Roem. & Schult., both with setaceous leaves.

Altogether 395 *Deschampsia* samples from 86 localities were included in this study (Fig. 1C; Supporting Information, Table S1). Generally, five individuals per locality were sampled, but in some cases fewer plants were available. Vouchers have been deposited in the herbarium of the University of Vienna (WU).

DNA EXTRACTION, RADSEQ AND PLASTID GENOME SEQUENCING

Total DNA was extracted from silica-dried leaves using the Invisorb Spin Plant Mini Kit (STRATEC Molecular GmbH, Berlin, Germany). The genomic DNA was then cleaned with the Nucleospin gDNA Clean-up Kit (Macherey-Nagel GmbH, Düren, Germany). A Qubit 3.0 Fluorometer with a dsDNA HS Assay Kit (Thermo-Fisher Scientific Inc, USA) were used to quantify the cleaned DNA. In general, 48 accessions were pooled together for one RADseq library. The libraries were prepared following the protocol detailed in Paun *et al.* (2016) with some modifications following Xue *et al.* (2021). The difference is that for tetraploids, double the amount of DNA was used than for the diploids. All RADseq libraries were then sequenced as paired-end 125-bp reads with inline-index indexing on an Illumina HiSeq 2500 at the Next Generation Sequencing Facility at Vienna BioCenter Core Facilities (VBCF), Austria.

One Next Generation Sequencing (NGS) library including 64 European individuals was further prepared for whole genome sequencing to extract the sequence of plastid genomes. We chose one sample from representative localities for the plastid library. The process of library preparation was performed using a TruSeq DNA PCR-Free Low Throughput Library Prep Kit (Illumina, USA) and followed the manufacturer's protocol. This library was sequenced as 50-bp paired-end reads on an Illumina HiSeq 2500 at the NGS Facility of VBCF.

PROCESSING OF RADSEQ DATA

The reads were firstly demultiplexed based on the index reads with BAMINDEXDECODER v.1.03, included in the Picard Illumina2Bam package (available from https://github.com/wtsi-npg/illumina2bam). The output bam files were converted to fastq files by picard v.2.18.26 (available from https://github.com/broadinstitute/picard/) and then processed with STACKS v.1.47 (Catchen *et al.*, 2013). Process_radtags was performed to clean reads by removing reads with an uncalled base or low scores, rescue barcodes and cut sites with a maximum of one mismatch.

In this study we used the published genome of *Hordeum vulgare* L. (GenBank assembly accession: GCA_004114815.1, Zeng *et al.*, 2020) as the reference. All fastq files of 395 samples were mapped to this reference using BWA v.0.7.12-r1039 (Li & Durbin, 2009) with default settings, and were then sorted, read groups were added and realigned around indels with SAMTOOLS v.1.6 (Li, 2011) and the GENOME ANALYSIS TOOLKIT v.3.8.1 (McKenna *et al.*, 2010).

DATA FILTERING, GENETIC STRUCTURE ANALYSIS

To identify the multidimensional structure in the RADseq data, we performed an overall analysis on the whole data (dataset 1) and three partial analyses as explained below (datasets 2, 3 and 4).

Dataset 1 (all 395 samples)

Genotype likelihoods were calculated with ANGSD v.0.929-24 (Korneliussen *et al.*, 2014) based on the realigned bam files. The settings were as follows: the gatk model (-GL 2), with a minimum nucleotide
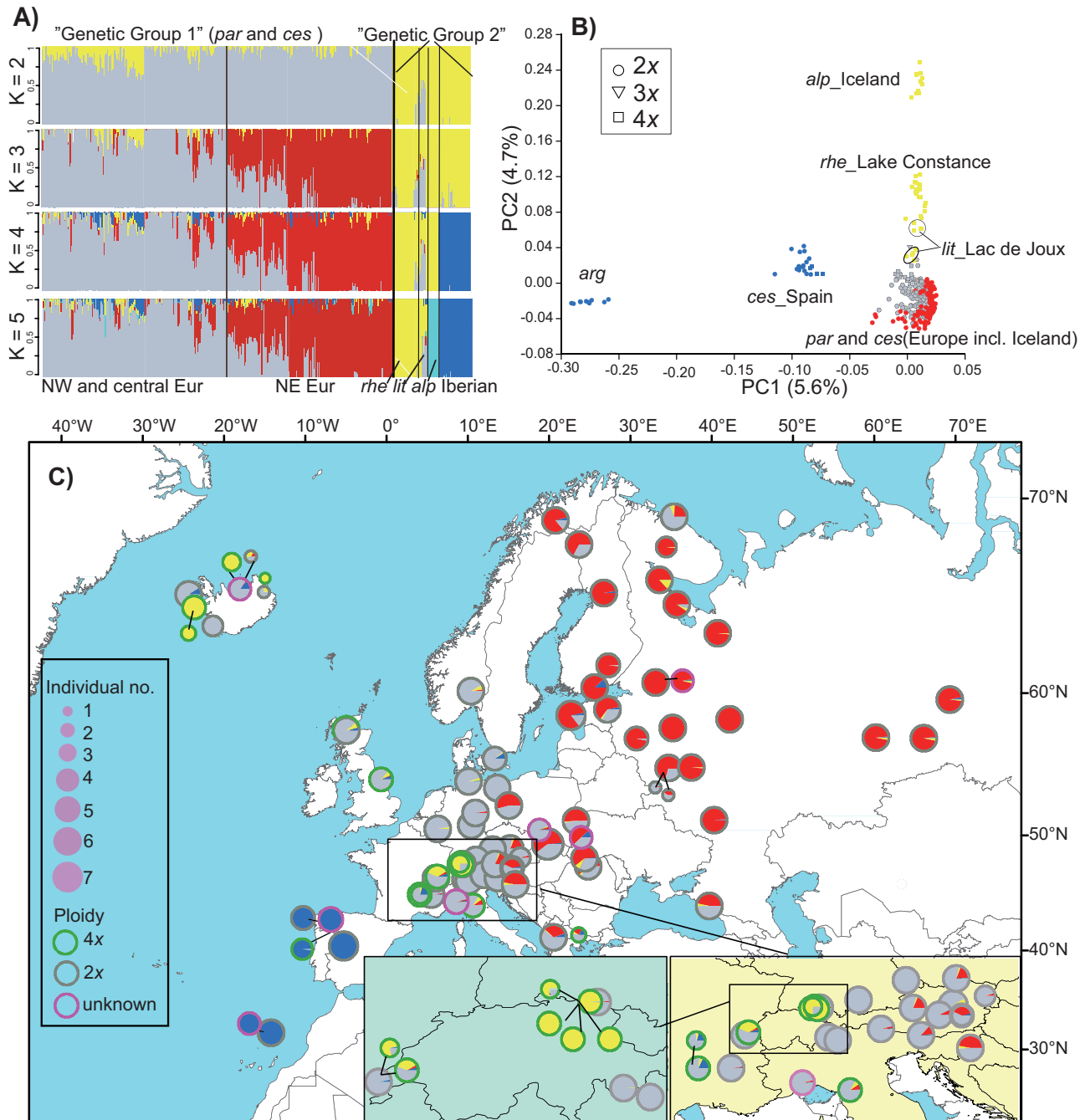
Figure 1. Clustering patterns of 395 accessions sampled across 86 localities in European *Deschampsia* (dataset 1). A, genetic structure and admixture based on 19 988 unlinked polymorphic sites as inferred with ANGSD. Admixture diagrams are shown for K = 2–5. Ancestry proportions inferred with NGSadmix are shown as vertical bars, where each bar represents an individual. B, PCA results based on 1 816 391 polymorphic sites from PCAngsd results. Genetic groups (K = 4) and ploidy are represented in different colours or shapes. Colours are according to K = 4 in (A). C, sampling map of *Deschampsia* sampling localities investigated in this study. Genetic structure and admixture (K = 4) based on localities are shown with colours on the map. Colours are according to (A) (K = 4). The map data were downloaded from the open-access website: diva-gis.org. Circle sizes represent the sample size. The colour of the ring represents different ploidy. Abbreviation: *ces*: subsp. *cespitosa*; *par*: subsp. *parviflora*; *rhe*: subsp. *rhenana*; *lit*: subsp. *littoralis*; *alp*: subsp. *neoalpina*; *arg*: *Deschampsia argentea*; Iberian: Spanish *D. cespitosa* subsp. *cespitosa* and the Macaronesian island endemic *D. argentea*; NW and central Eur: north-western and central Europe; NE Eur: north-eastern Europe.

and mapping quality of 20 (-minQ 20, -minMapQ 20), retaining only variable positions with a high likelihood (SNP_pval 1e-6), with data for at least 50% of the samples, and alleles present in a minimum of four samples to generate a beagle variant file. Although developed for diploid data, the genotype-free method of ANGSD was suggested to retain allele dosage information more accurately within genotype likelihoods for polyploid samples as compared to standard approaches (Záveská *et al.*, 2019).

To assess relationships among all samples, ordinations were calculated by PCAngsd, which is especially suitable for low to medium depth sequencing samples (Meisner & Albrechtsen, 2018), based on genotype likelihoods from the output beagle file and plotted with R v.3.2.3 (R Core Team, 2015) in RSTUDIO v.1.1.463 (RStudio Team, 2015) with ggplot2 v.3.2.1 (https://ggplot2.tidyverse.org). The beagle file was then filtered to retain one variant every 10 000 bp as unlinked sites, which were then used for admixture analyses. We used NGSadmix to run each K (from 1 to 12) for ten independent runs starting from different seeds. The optimal value of K was selected by calculating ΔK with the Evanno method (Evanno *et al.*, 2005) in CLUMPAK (Kopelman *et al.*, 2015). The final admixture plots were visualized in R.

To avoid bias from highly different sizes of genetic groups, we performed an additional analysis. We chose a smaller but more balanced set of samples in each genetic group of dataset 1 based on the above admixture results and included 147 samples. We then calculated the covariance matrix as above, which was plotted as PCA and a heatmap with the R package Gplots v.3.1.1 (http://CRAN.R-project.org/package=gplots) in R. The admixture analysis was done using the same method as above.

Dataset 2 (Genetic Group 1 of dataset 1, including 323 samples)

According to the admixture result of K = 2 of dataset 1, we focused on the first group (only *D. cespitosa* subspp. *cespitosa* and *parviflora*) and calculated the genotype likelihoods by ANGSD again, with the same filter setting as for dataset 1. The covariance matrix was calculated by PCAngsd as before. The PCA result was plotted in R. The beagle SNPs file was then filtered and used for the admixture analysis in the program NGSadmix (K from 1 to 12) for ten independent runs. The optimal value of K was selected, and the final admixture best K plots were visualized in R with the same methods.

Dataset 3 (all diploids)

The PCA and admixture analyses on the diploid accessions only were performed using the above method. Nucleotide diversity and absolute divergence estimates were calculated based on the genotype likelihoods in ANGSD. We used Watterson's estimator (theta) and Tajima's estimator (pi) to represent nucleotide diversity and showed them for each locality on a map. For this analysis, only localities with five or more samples (for the latter we down-sampled the data to consistently use five samples per locality) were included. Based on the realigned bam files, we calculated site allele frequency likelihood (-dosaf 1) and then estimated the maximum likelihood of folded site frequency spectra (SFS) using the program realSFS in ANGSD (-fold 1). Genome-wide diversity was then calculated using the program *thetaStat* and plotted in R. To estimate divergence F_{ST} between localities, we firstly used ANGSD to calculate saf files and then used realSFS to estimate 2d-SFS for each pair. Then the differentiation between each pair was estimated with F_{ST} in the program realSFS based on the 2d-SFS and all the safs. We then averaged the F_{ST} of sites across 10-kb windows.

Finally, we calculated the number of private alleles for localities with five samples, which can provide an estimate of the relative age of different populations or lineages. Ref_map.pl in STACKS was performed to call the genotypes based on the realigned bam files using the default settings, and the Populations program in STACKS was used to calculate the number of private alleles for each locality.

Dataset 4 (all tetraploids)

To assess the relationships among tetraploid localities and subspecies, we estimated genotype likelihoods in ANGSD including 68 samples from 18 localities. We used similar filter settings as for dataset 1 (-GL 2, -minQ 20, -minMapQ 20, SNP_pval 1e-6, with data for at least 50% of the samples) to generate a beagle variant file, with alleles present in two or more samples. Firstly, a PCA analysis was performed with PCAngsd as before. Based on the covariance matrix from PCAngsd, a heatmap was plotted. Finally, the admixture analysis was done using a thinned, unlinked dataset.

PLASTID DATA ASSEMBLY, FILTERING AND ANALYSIS

Whole-genome data was demultiplexed based on index reads, allowing for a maximum of one mismatch with the program BAMINDEXDECODER. TRIMMOMATIC v.0.36 (Bolger *et al.*, 2014) was used to filter the raw reads applying the option ILLUMINACLIP to remove the adapters. Since most samples had a coverage insufficient for reliable *de novo* assembly, we performed a mapping-based pipeline (Heckenhauer *et al.*, 2018) combining BWA and GATK HaplotypeCaller with ploidy set to one to extract the

plastid genome SNP information as a vcf file, then replaced the reference with the SNP information with FastaAlternateReferenceMaker in GATK. For this analysis, we used *D. cespitosa* (NC_040999) as the plastid reference. We then inferred a phylogenetic tree with RAxML v.8 (Stamatakis, 2014) using 1000 rapid bootstrap replicates and the GTRGAMMA model. We included four outgroup species (*Deschampsia tenella* Petrie, *Deschampsia chapmanii* Petrie, *Deschampsia gracillima* Kirk and *D. antarctica*) from the Southern Hemisphere in the whole plastid genome alignment. The RAxML tree was rooted with the southern clade according to our earlier results (Xue *et al.*, 2021), and visualized with FIGTREE v.1.4.4 (https://github.com/rambaut/figtree/). Finally, we used the library pegas in R to determine the plastid haplotypes and build a haplotype network (Paradis, 2010).

RESULTS

RADSEQ RESULTS

After demultiplexing and filtering the raw reads, our data contained on average 4.66 million high-quality reads per diploid individual and 4.86 million reads per polyploid individual. These data have been deposited in the NCBI Short Reads Archive (BioProject ID PRJNA887944, SRA Study ID SAMN31204683–SAMN31205077). The mapping rates of diploid and tetraploid reads to the *Hordeum vulgare* reference were 36% and 32%, respectively. For dataset 1, after filtering the beagle file, we retained 1 816 391 variable sites. For the later admixture analysis, after thinning the variants every 10 000 bp, we kept only 19 988 in the unlinked beagle file. For the smaller dataset 1, the filtered beagle file retained 1 479 184 variable sites, and the unlinked file included 20 092 sites. For dataset 2, the direct-estimation from ANGSD kept 1 549 513 sites and the unlinked beagle retained 19 792 variable sites for the admixture analysis. For dataset 3, we kept 1 564 633 sites from ANGSD and 19 773 unlinked sites. For dataset 4 (68 tetraploids), the filtered output beagle file included 906 974 variable sites and the unlinked file included 19 946 sites.

ENTIRE EUROPE PATTERNS (DATASET 1)

By evaluating the log probability values from different admixture runs using the Evanno method, the separation into three groups had the highest ΔK value (Supporting Information, Fig. S1A). The next higher peaks (K = 2 and K = 4, 5) were also visualized (Fig. 1A). K = 2 can clearly differentiate the diploids of *D. cespitosa* subspp. *cespitosa* and *parviflora* and the seminiferous tetraploids (only *D. cespitosa* subsp. *cespitosa*), hereafter referred to as 'Genetic Group

1', from 'Genetic Group 2' including the remaining samples: pseudoviviparous tetraploids (including *D. cespitosa* subspp. *neoalpina* and *rhenana*), the seminiferous tetraploid *D. cespitosa* subsp. *littoralis* and all Iberian populations (Fig. 1A). With K = 3, the first genetic cluster of K = 2 was partitioned into two clusters: (1) north-western and central Europe, (2) north-eastern Europe (Fig. 1A). With K = 4, the 'Iberian Group' of both diploid and tetraploid *D. cespitosa* subsp. *cespitosa* in Spain and diploid *D. argentea* endemic to Macaronesia was further separated, which is also consistent with the results of the PCA (Fig. 1A, B). To better estimate the genetic structure, we assigned all the individuals of the 86 localities to the four clusters of K = 4. These were visualized as pie charts on the map (Fig. 1C). The results for all Ks showed *D. cespitosa* subsp. *littoralis* with substantial admixture from *D. cespitosa* subsp. *cespitosa*.

The ordination plot from PCAngsd clearly separated several groups (Fig. 1B). The components PC1 and PC2 accounted for 5.6% and 4.7% of the total variation, respectively. The first axis clearly separated the Macaronesian island endemic *D. argentea* and the Spanish *D. cespitosa* subsp. *cespitosa* from the remaining individuals. The second axis further separated some polyploids, including *D. cespitosa* subspp. *neoalpina*, *rhenana* and *littoralis* from the remaining *D. cespitosa*. In detail, *D. cespitosa* subsp. *neoalpina* samples were the most different from other *D. cespitosa* samples, even from those co-occurring in Iceland. The individuals of *D. cespitosa* subsp. *rhenana* were between *D. cespitosa* subsp. *neoalpina* and the large *D. cespitosa* group; one locality of *D. cespitosa* subsp. *littoralis* was included in the *D. cespitosa* subsp. *rhenana* group, whereas samples from another locality were found closer to the large *D. cespitosa* group. For the remaining large group, there was a slight geographical pattern to be seen from central-western to eastern-northern Europe, whereas other polyploids were nested in the diploids.

Analysing a smaller but more balanced selection regarding sample size (147 individuals) of dataset 1 yielded similar results for the PCA (Supporting Information, Fig. S2B) compared to the whole dataset (Fig. 1B), but with a more pronounced separation of *D. argentea*. Both axes explained *c.* 9.2% of the total variation. The heatmap (Supporting Information, Fig. S2A) complemented the PCA results well, pointing to five clear genetic groups (Genetic Group 1, *rhenana*, *neoalpina*, Spanish *cespitosa* and *D. argentea*). The heatmap also showed *D. cespitosa* subsp. *littoralis* sharing higher co-ancestry with *D. cespitosa* subspp. *cespitosa* and *rhenana* than with other groups. Further, Spanish *D. cespitosa* showed high co-ancestry with both Genetic Group 1 and *D. argentea*. Admixture results for this smaller dataset (Supporting Information, Fig. S2C)

were slightly different from the admixture results of the entire sampling, which indicated that large differences in group size affect the admixture results. Highest ΔK was found for two groups (Supporting Information, Fig. S1B), which already separated some polyploids from all other localities, with *D. cespitosa* subsp. *littoralis* showing admixture between these two groups. K = 3 could further separate the Iberian Group. A relatively high ΔK was also found for K = 6 (*cespitosa*1, *cespitosa*2, *littoralis* and *rhenana*, *neoalpina*, Spanish *cespitosa*, *argentea*).

DATASET 2 (GENETIC GROUP 1 OF DATASET 1)

To study the genetic structure of *D. cespitosa* in Europe in detail, we excluded the divergent Genetic Group 2 (including the pseudoviviparous tetraploids *D. cespitosa* subspp. *neoalpina* and *rhenana*, the seminiferous tetraploid *D. cespitosa* subsp. *littoralis* and the Iberian Group). The admixture analysis yielded K = 5 (Supporting Information, Fig. S1C) as the best solution corresponding to the finest geographical patterns. Four of the five genetic groups showed in

part substantial admixture from other groups (Fig. 2A, B). The five genetic clusters were as follows: (1) Iceland and western Scandinavia, including one locality of Murmansk; (2) western central Europe; (3) central Europe with the Alps; (4) eastern central Europe extending to the north in eastern Scandinavia; and (5) eastern Europe (western Russia) including three trans-Ural localities. The PCA showed a fuzzy overlapping structure due to several localities found in other clusters (Fig. 2C). The first axis explained 3.7% of the variation, showing a rough geographical pattern from west to east, whereas the second axis explained 1.5% of the variation and revealed, in part, a pattern from north (Iceland) to south (Italy).

DATASET 3 (ALL DIPLOIDS)

The separation in three groups was supported by the highest ΔK values other than two (Supporting Information, Fig. S1D); the other Ks are also informative and the genetic groups are similar to results from dataset 2, but include the Iberian diploid localities (missing in dataset 2). The corresponding admixture
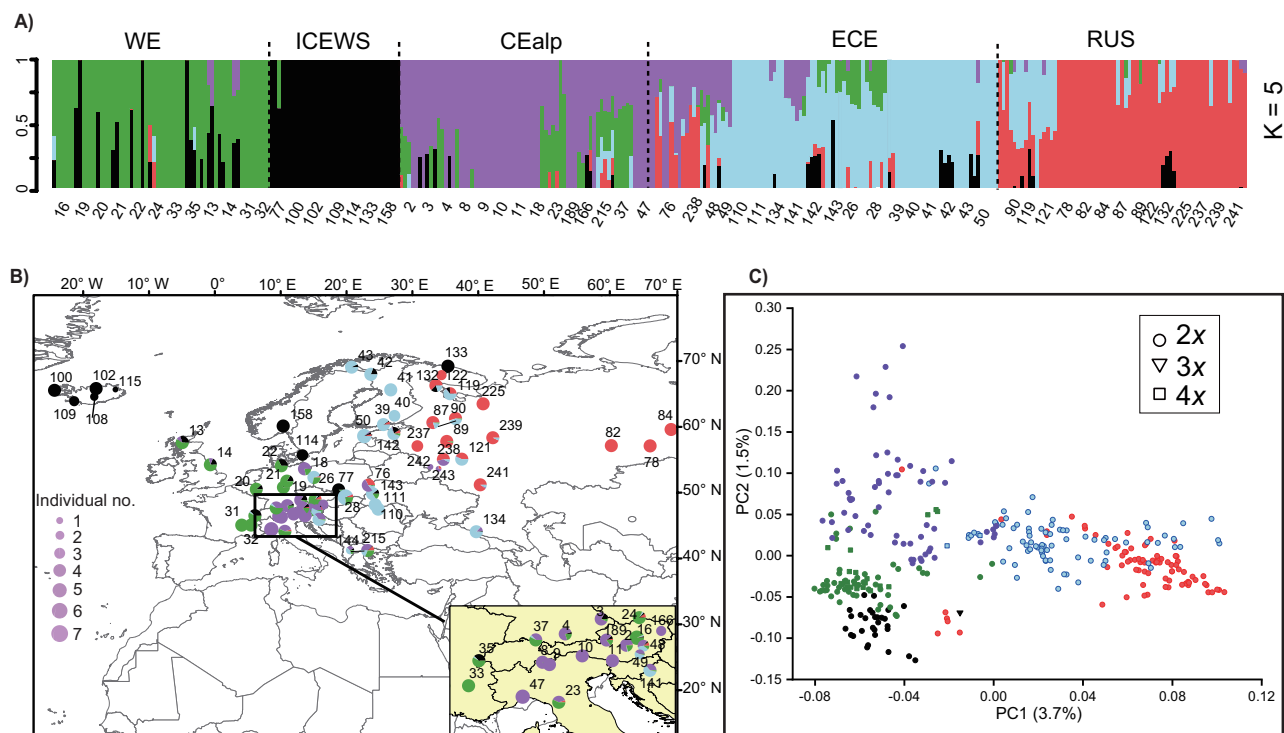

Figure 2. Clustering patterns of 323 *D. cespitosa* accessions from the 'Genetic Group 1' of dataset 1 (dataset 2: red and grey part of dataset 1). A, genetic structure and admixture based on 19 793 unlinked polymorphic sites as inferred with ANGSD. An admixture diagram is shown for K = 5. B, map showing admixture (K = 5) based on localities. Colours are according to (A). C, PCA results of 323 accessions based on 1 549 513 polymorphic sites from PCAngsd results. Genetic groups (K = 5) are represented in different colours, colours are according to (A). Ploidy is represented by different shapes. Abbreviations: WE: western central Europe; ICEWS: Iceland and western Scandinavia; CEalp: central Europe with the Alps; ECE: eastern central Europe extending to the north in eastern Scandinavia; RUS: eastern Europe (western Russia including three trans-Ural localities).

results were also shown on the map (Supporting Information, Fig. S3A). The PCA (Supporting Information, Fig. S3B) showed similar results with the reduced dataset 1. The first axis explained 5.7% of the variation and separated the Iberian Group, and the second axis explained 3.7% of the whole variation and revealed a faint geographical west-east structure.

Estimates of nucleotide diversity (mean pi and theta of each locality based on the five genetic groups of dataset 2 and the Iberian group) are shown in Figure 3A, B and are plotted on a map based on each locality (Fig. 3C). The average diversity was low for most localities (theta: 0.008–0.013; pi: 0.008–0.015), but several localities in central Europe and eastern Europe showed higher genetic diversity, suggesting admixture.

The number of private alleles of each locality is shown in Figure 3D. Localities of northern latitudes and high mountains exhibited a lower number of private alleles.

Localities in Madeira, Spain and periglacial localities in the Alps showed a higher number of private alleles.

Pairwise F_{ST} values between localities were used for comparing the peculiar location 133 of the Murmansk region with those of Iceland and western Scandinavia (belonging to the same K5 group) and other localities of the four remaining genetic groups and the Iberian Group. Lowest pairwise F_{ST} values were found between Icelandic localities (pop102 and 158) and Murmansk locality 133 (mean F_{ST}: pop133 versus 102: 0.057; pop133 versus 158: 0.059) (Supporting Information, Fig. S4).

TETRAPLOIDS

For dataset 4, we used the covariance matrix from PCAngsd to perform the heatmap and PCA analyses. The heatmap (Fig. 4A) showed the seven tetraploid and seminiferous *D. cespitosa* localities from the United
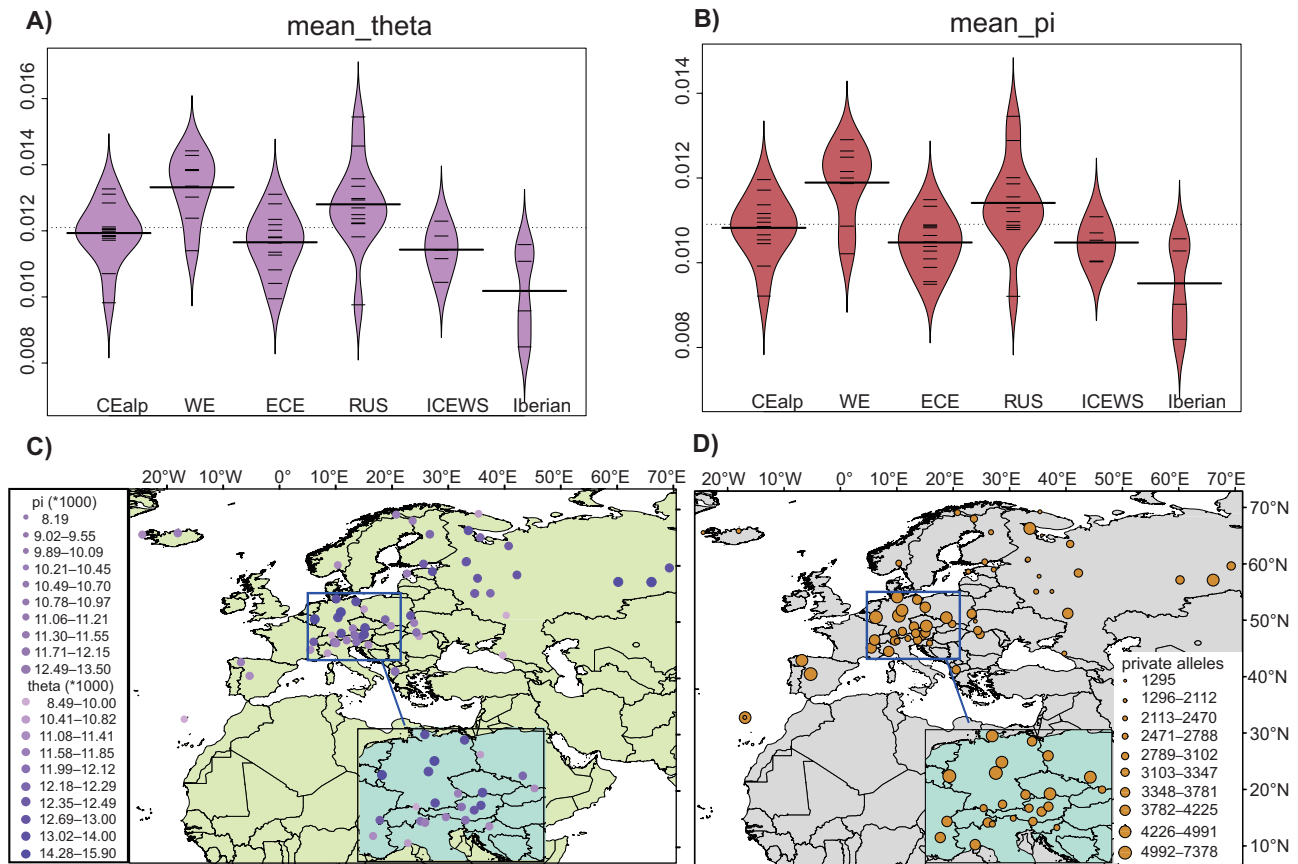

Figure 3. A, bean plot of nucleotide diversity (mean theta) of each locality. B, bean plot of nucleotide diversity (mean pi of each locality). C, nucleotide diversity of each locality shown on the map, with colour intensity representing theta and circle size representing pi. D, private allele numbers based on the localities estimated from the Populations program in STACKS. Circle size represents the number of the alleles. Abbreviations: CEalp: central Europe with the Alps; WE: western central Europe; ECE: eastern central Europe extending to the north in eastern Scandinavia; RUS: eastern Europe (western Russia including three trans-Ural localities); ICEWS: Iceland and western Scandinavia; Iberian: Spanish *D. cespitosa* subsp. *cespitosa* and the Macaronesian island endemic *D. argentea*.

Kingdom, France, Spain and Macedonia sharing higher co-ancestry. In the former analyses of dataset 1 and 2 (Figs 1, 2) they share higher co-ancestry with diploids in the same region. Two localities of seminiferous *D. cespitosa* subsp. *littoralis* show higher co-ancestry with both seminiferous *D. cespitosa* subsp. *cespitosa* and pseudoviviparous *D. cespitosa* subsp. *rhenana*. One locality of *D. cespitosa* subsp. *rhenana* is more similar to *D. cespitosa* subsp. *littoralis* than to the other *D. cespitosa rhenana* localities. The four localities of pseudoviviparous *D. cespitosa* subsp. *neoalpina* are different to all other subspecies. The first axis of the constructed PCA (Fig. 4B) explained 3.8% of the total variation, which separated *D. cespitosa* subsp. *neoalpina*. The second axis (explaining 1.7%) separated five localities of *D. cespitosa* subsp. *rhenana* from all *D. cespitosa* localities. One locality of *D. cespitosa* subsp. *littoralis* was close to the *cespitosa* group, whereas another *littoralis* locality appeared between *rhenana* and *cespitosa*. The admixture analysis (Fig. 4C) found K = 3 (Supporting Information, Fig. S1E) as the best solution confirming the above results with one locality of each *littoralis* and *rhenana* being intermediate.

PLASTID DATA

After demultiplexing the raw reads, high-quality reads in our NGS data ranged from 5.1 to 17.5 million per individual, with an average of 10.0 million. The WGS raw data have been also deposited in the NCBI Short Reads Archive (BioProject PRJNA886965, SRA accessions SAMN31148700–SAMN31148763). Only 24 708–159 858 (average 78 006) pairs could be mapped to the reference. After mapping the filtered reads of 64 individuals to the published plastid reference, the average coverage ranged from 10 × to 60 × (average coverage 30 ×). After adding the outgroups, the length of the final alignment matrix was 135 783 bp, with 495 characters that were potentially informative. The best-scoring ML tree with bootstrap support from the RAxML analysis is shown in Figure 5A. There are three strongly supported major clades: a widespread northern (green) clade; an essentially central European clade (red) including a south-eastern group; and a smaller group of haplotypes (blue) occurring in the highly differentiated mostly pseudoviviparous tetraploids and a few diploids. A haplotype network based on the SNP dataset is presented in Figure 5B. The haplotype network analysis showed that the 64 plastid SNP sequences formed 57 haplotypes. The three haplotype groups revealed by the ML tree and the haplotype network are shown on the map (Fig. 5C).

DISCUSSION

BIOGEOGRAPHIC STRUCTURE

The structure revealed in the entire RADseq dataset provided evidence for strong divergence of tetraploid
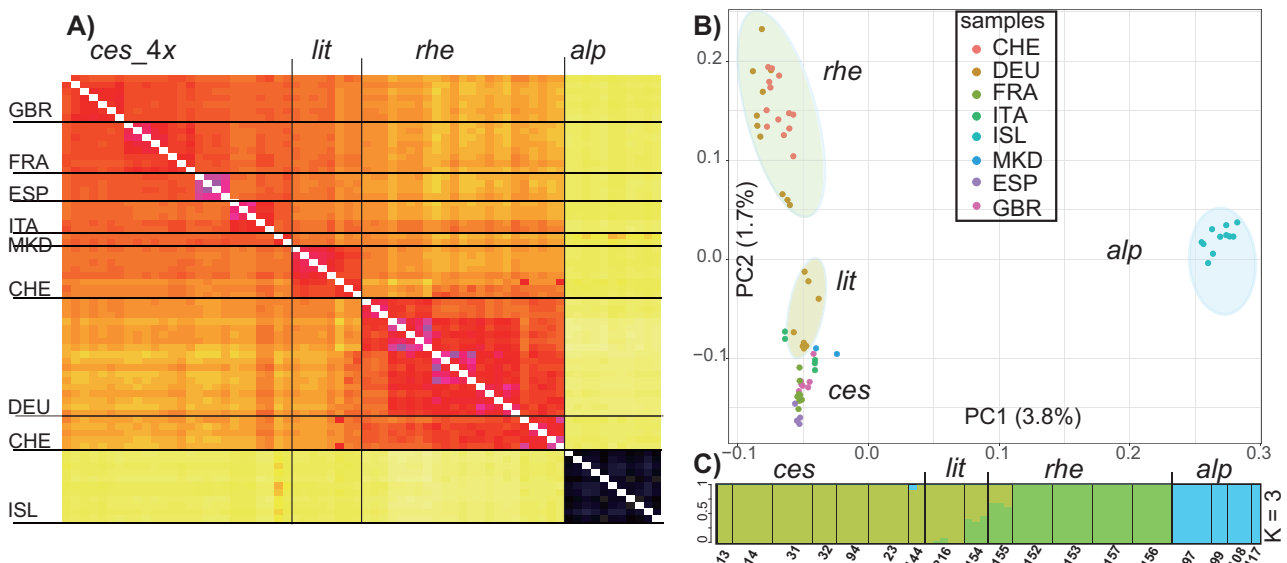

Figure 4. Clustering patterns of 68 accessions from 18 localities of tetraploid *D. cespitosa* (dataset 4). A, heatmap obtained with covariance matrix results from PCAngsd. Region information is on the left and subspecies names are on the top. B, PCA results based on 906 974 polymorphic sites from PCAngsd. Subspecies and localities are represented in different colours. C, genetic structure and admixture based on 19 947 unlinked polymorphic sites from ANGSD. Admixture diagrams show K = 3. Locality information is at the bottom and subspecies names are on the top. Abbreviations: *ces*: subsp. *cespitosa*; *rhe*: subsp. *rhenana*; *lit*: subsp. *littoralis*; *alp*: subsp. *neoalpina*; GBR: Great Britain; FRA: France; ESP: Spain; ITA: Italy; MKD: North Macedonia; CHE: Switzerland; DEU: Germany; ISL: Iceland.
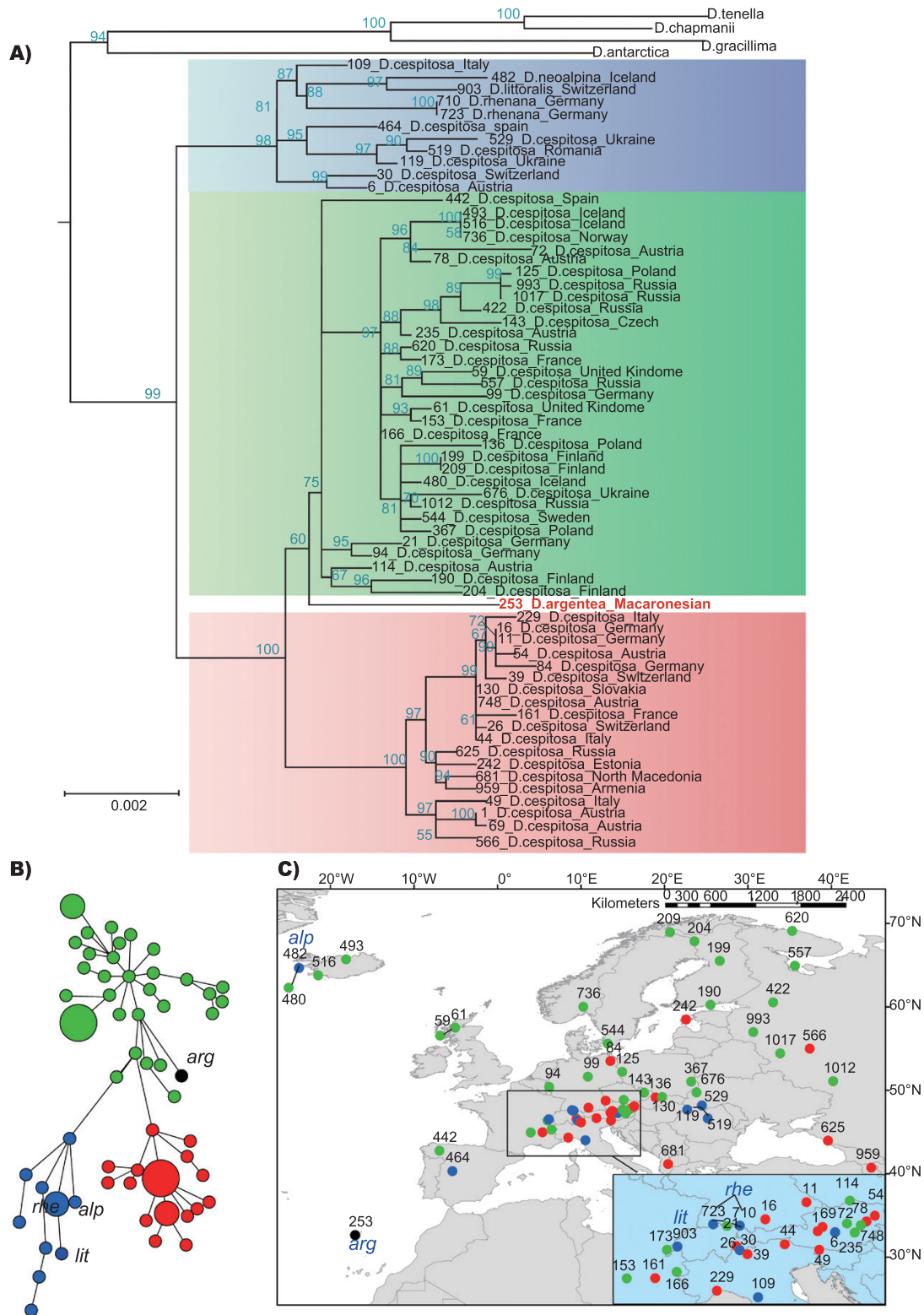
Figure 5. Results of phylogenetic inference based on plastid data. A, the RAxML tree based on 357 SNPs from 64 individuals. Numbers on each branch are bootstrap support. The tree was rooted with taxa from the Southern Hemisphere, including *D. tenella*, *D. chapmanii*, *D. gracillima* and *D. antarctica*. B, haplotype network from Pegas with 57 haplotypes. C, the three clusters from the ML tree shown on the map. Abbreviations: *rhe*: subsp. *rhenana*; *lit*: subsp. *littoralis*; *alp*: subsp. *neoalpina*; *arg*: *Deschampsia argentea*.

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D. cespitosa subsp. *neoalpina* of Iceland, *D. cespitosa* subsp. *littoralis* (Swiss Jura mountains), *D. cespitosa* subsp. *rhenana* (Lake Constance) and the Iberian Group including Spanish *D. cespitosa* subsp. *cespitosa* (diploid and tetraploid) and the Macaronesian diploid *D. argentea* (Fig. 1A, yellow, Genetic Group 2). All remaining European samples (Genetic Group 1) clustered in only two genomic groups, namely a north-western and central European Group (Fig. 1C, grey) and a north-east European Group (Fig. 1C, red). The higher numbers of private alleles of the continental Spanish *D. cespitosa* subsp. *cespitosa* and Macaronesian *D. argentea* (Fig. 3D) may confirm the role of the Iberian Peninsula as a refugial area and the Pyrenees as a barrier to the dispersal for some organisms (Hewitt, 1999; Gómez & Lunt, 2007; Miraldo *et al.*, 2011; Pinto-Carrasco *et al.*, 2022). However, in our sample we were not able to include populations between Spain and the Alps as the samples of Massif Central were found to be polyploid and were therefore excluded from this analysis. The Iberian populations show low genetic diversity (Fig. 3A-C) consistent with having experienced genetic bottlenecks in refugia during the glacial period and host private alleles indicating long-term isolation.

Strong divergence of Iberian populations has also been observed in other widespread grassland plants such as *Festuca rubra* L. (von Cräutlein *et al.*, 2019). In *Anthoxanthum odoratum* L., the Spanish populations appeared well isolated from central Europe, but were connected to south-western Scandinavia pointing to an Atlantic western European colonization route between these regions (Pimentel *et al.*, 2007). In the *Hordeum murinum* L. group, Bieniek (2018) found some nuclear SSR genotypes and plastid DNA haplotypes restricted to Spain.

In contrast to the RADseq data, the pattern within and among plastid haplotype groups of *D. cespitosa* revealed a poor structure similar to that observed in other widespread grasses, e.g. *Phalaris arundinacea* L. (plastid SNP: Perdereau *et al.*, 2017), *Nardus stricta* L. (Zorj, 2013), *Poa pratensis* L. (Raggi *et al.*, 2015) and the sedge *Carex nigra* (L.) Reichard (Jiménez-Mejías *et al.*, 2012). Poor genetic structure, however, can result from different backgrounds: (1) in the genetically highly variable nearly panmictic superpopulations of outcrossers such as *P. arundinacea* (Perdereau *et al.*, 2017), *C. nigra* (Jiménez-Mejías *et al.*, 2012) and *D. cespitosa* (this study); and (2) at the other extreme of mostly clonally reproducing plants such as the genetically uniform *N. stricta* (Zorj, 2013) with low levels of genetic diversity. Among the natural factors, wind pollination is an important agent promoting gene flow in outcrossing and genetically polymorphic *D. cespitosa*. Likewise, the poor genetic structure on

a European scale found in the ecologically similar but more restricted *C. nigra* was attributed to wind pollination and multiple secondary contacts in the nearly continuous range of this sedge (Jiménez-Mejías *et al.*, 2012).

There is a faint geographical structure in our whole plastid genome data of *D. cespitosa*, indicated by RAxML analyses. The widespread green haplotype group occurs in central and northern Europe including most diploids and one tetraploid of *D. cespitosa* subsp. *cespitosa*. The red group, including most diploids and one tetraploid *D. cespitosa* subsp. *cespitosa* in central and southern Europe, and the blue haplotype group, containing all tetraploids of *D. cespitosa* subspp. *littoralis* and *rhenana* and several diploids and one tetraploid of *D. cespitosa* subsp. *cespitosa*, are scattered across central and southern Europe, however, including *D. cespitosa* subsp. *neoalpina* from Iceland (Fig. 5C). The strong support for the blue and the red group indicates that these are singular lineages of which one (the red) may have been the source of the green group providing the recolonizing plants for the northern regions after the retreat of the ice sheets.

The pattern identified by the plastid data in part concurs with the K = 3 partition of the entire RADseq data (Fig 1A). The pseudoviviparous tetraploids (*D. cespitosa* subspp. *neoalpina* and *rhenana*) and the seminiferous tetraploid *D. cespitosa* subsp. *littoralis* appear in a strongly supported haplotype group, but with another seminiferous tetraploid and a few diploids of *D. cespitosa* subsp. *cespitosa*. The distribution of the green (+/- northern and eastern Europe) and red haplotypes (+/- central Europe) roughly follows the split in the RADseq data, however, extending far into north-western Europe. Highly resolving plastid data often provide detailed information on genetic structure in recolonized regions (Perdereau *et al.*, 2017; von Cräutlein *et al.*, 2019). Incongruence between plastid and nuclear markers, as found in part in our study, can result from several reasons such as incomplete lineage sorting or deep reticulation. In fact, diploid *D. cespitosa* s.l. (mostly $2n = 26$: Kawano, 1963; Rothera & Davy, 1986) was assumed to result from ancient allopolyploidization based on karyotype structure (Garcia-Suarez *et al.*, 1997), whereas the seminiferous tetraploids (mostly $2n = 52$: Kawano, 1963; Rothera & Davy, 1986) appear to have originated from multiple autopolyploidization according to our RADseq data that allowed a deeper insight into the genetic structure of European *D. cespitosa*.

After excluding the Iberian populations of diploid *D. argentea* and *D. cespitosa* (diploid and tetraploid) and the highly differentiated tetraploids (*D. cespitosa* subspp. *neoalpina, littoralis* and *rhenana*: see discussion in a separate paragraph below) in the RADseq data, the higher resolution in the structure

among the remaining diploid and seminiferous tetraploids (dataset 2, Genetic Group 1) revealed five geographical clusters in part with substantial genetic admixture from other clusters (Fig. 2): (1) western central Europe (WE); (2) Iceland and western Scandinavia (ICEWS); (3) central Europe with the Alps (CEalp); (4) eastern central Europe extending to the north in eastern Scandinavia (ECE); and (5) eastern Europe (RUS). No genetic support was found for the morphologically slightly different diploid *D. cespitosa* subsp. *parviflora* or the seminiferous tetraploid *D. cespitosa* subsp. *cespitosa*. In the Pleistocene, *Deschampsia* was probably pushed southwards by the Scandinavian ice sheet in western and central Europe, whereas in the north-eastern regions the conditions were more favourable, with even mountain conifers and deciduous trees surviving during the Last Glacial Maximum [(LGM): Tzedakis *et al.*, 2013]. Comparisons with similar studies on widespread and common grass species on an entire European scale are however limited due to different sampling design or density. In the study on *Festuca rubra*, von Cräutlein *et al.* (2019) found three major genetic groups: southern (Iberian); north-eastern (Scandinavian); and north-western (Iceland, Greenland). Central and eastern Europe were not included in their study.

PATTERNS OF (RE)COLONIZATION

In our study we found higher levels of genetic diversity and private alleles in the periglacial regions and in part in the Alps compared to the northern regions (Fig. 3). The genomic structure indicates (re-)colonization of formerly glaciated northern Europe and Iceland from several sources (refugia) in western, central and eastern Europe. Although phylogeographic history is in large part idiosyncratic for each taxon (Taberlet *et al.*, 1998), a colonization of Iceland, Britain and Ireland and western Scandinavia obviously from western central Europe (black and green localities in Fig. 2A, B) is more-or-less congruent with an Atlantic western European colonization route, as observed by Pimentel *et al.* (2007) in the grass *Anthoxanthum odoratum*. The Alps may have been recolonized twice: (1) plants of populations sitting south (light blue localities in Fig. 2A, B) of the high mountain ranges probably migrated around the retreating ice sheets east- and northwards, finally ascending to higher elevations from all sides; and (2) the Western Alps and the Jura Mountain range were colonized by more southern plants from western central Europe. Some of those made their way farther to the east. This pattern is reminiscent in part to what has been observed in *Helianthemum nummularium* Mill. *s.l.* (Volkova *et al.*, 2016), *Abies alba* (Aiton) Michx. (Konnert & Bergmann, 1995) and *Populus tremula* L. (Rendón-Anaya *et al.*, 2021). In

these species, Scandinavia was colonized from Eastern and Central European populations or eastern Europe (Russia), a pattern that seems to be more common (Hewitt, 1999).

POLYPLOIDS

The RADseq data (admixture, PCA) showed the seminiferous tetraploid *D. cespitosa* mostly in the regional clusters together with the closest diploid populations (Fig. 1A, B). This pattern, with the occurrence of diploids and tetraploids at the same locality, as at locality P13 in the United Kingdom, or geographically close localities, like P93 and P94 in Spain, points to multiple autopolyploid formations of those tetraploids. Notable admixture in several of those localities may point to sexual autotetraploid formation between genotypes, probably via a triploid bridge which is considered the primary pathway in the formation of polyploids (Spoelhof *et al.*, 2017).

The genetic structure as revealed by K = 3 and 4 (RADseq: Fig. 1A, B) and their similar plastid haplotypes may indicate closer relationships among the pseudoviviparous periglacial lineages (*D. cespitosa* subsp. *neoalpina* and *rhenana*) and seminiferous *D. cespitosa* subsp. *littoralis*. The genetic structure only based on the tetraploid samples revealed that *D. cespitosa* subsp. *neoalpina* separated first and shared lower co-ancestry, whereas *D. cespitosa* subspp. *rhenana* and *littoralis* shared higher co-ancestry with *D. cespitosa* subsp. *cespitosa* (Fig. 4A, B). However, the origin of these taxa is still unclear. Regarding *D. cespitosa* subsp. *neoalpina*, we failed to sample additional populations from other regions than Iceland for testing the polyphyly hypothesis of earlier authors (Hedberg, 1958; Kawano, 1963), assuming that isolated northern subarctic lineages gave rise to pseudoviviparous variants in different regions. From our data, one could assume a periglacial survival of an ancestor of *D. cespitosa* subspp. *neoalpina* and *rhenana* becoming disjunct in the post-glacial period. The periglacial lineage *D. cespitosa* subsp. *rhenana* may have adapted in the post-glacial or an interglacial to the changing flooding regime on the shores around Lake Constance, like other pre-Alpine endemics of this region (Peintinger *et al.*, 2012). However, these lineages must have diverged from their ancestors in some peri-Alpine isolation as the lakes were covered by a huge ice sheet during the LGM (Van Husen, 1987). Another case of adaptation to a special flooding regime with substantial genetic differentiation but low morphological separation from co-occurring *D. cespitosa* subsp. *cespitosa* was found in *D. cespitosa* subsp. *wibeliana* in the Elbe Estuary of northern Germany (Heydel *et al.*, 2017). These diploid plants

(Albers, 1975) growing under conditions of tidal flooding are considered neo-endemics in the process of ecological speciation (Heydel *et al.*, 2017).

Another tetraploid but seminiferous variant either included in *D. cespitosa* subsp. *rhenana* (Chiapella, 2000) or vice versa (*rhenana* included in *littoralis*: Conert, 1987) is confined to lake shores in the Swiss Jura Mountains (Peintinger *et al.*, 2012). The two *littoralis* localities of Lac de Joux show a hybrid pattern between *D. cespitosa* subspp. *rhenana* and *cespitosa* in the heatmap and admixture analysis (Fig. 4A, C). PCA analysis further confirmed that one locality of subsp. *littoralis* is closer to subsp. *cespitosa* while the other locality is intermediate between *D. cespitosa* subspp. *cespitosa* and *rhenana* (Fig. 4B). This may result from a scenario of an ancient wider distribution of a periglacial variant, the only remaining descendants of which are now widely separated and of which one has become pseudoviviparous (*D. cespitosa* subsp. *rhenana*) at some time and the other (*D. cespitosa* subsp. *littoralis*) has remained seminiferous with strong introgression at present from *D. cespitosa* subsp. *cespitosa*.

PAST AND PRESENT NATURAL AND HUMAN IMPACT ON THE BIOGEOGRAPHY OF *D. CESPITOSA*

Besides the regional groups identified in *D. cespitosa*, we see poor resolution over large regions. Both natural (especially animal dispersal: Poschlod & Bonn, 1998) and increasingly important anthropogenic factors for dispersing propagules over long distances and continents (Whinam *et al.*, 2005) may have acted in concert to produce the present distribution patterns in several plants. This may be true also for the observed patterns in *Deschampsia*. Multiple secondary contacts as assumed for the poor genetic structure in *C. nigra* (Jiménez-Mejías *et al.*, 2012) are even more likely in *D. cespitosa*, given its broader ecological niche and its wide elevational amplitude. In the open early post-glacial tundra landscape, wind was also an important seed and pollen dispersal vector, but in the later woody landscape animal dispersal of seeds (epi- and endo-zoochoric) was more effective over long distances (Poschlod & Bonn, 1998). Plant dispersal patterns are assumed to have changed fundamentally with the decline of the large browsing animals since the beginning of the Neolithic and increasing human impact on landscape and vegetation (Poschlod & Bonn, 1998). Today *D. cespitosa* is a prominent weed in mountain pastures, favoured by soil compaction, and it thrives in fallow land (Rosenthal & Hölzel, 2009). It often becomes dominant after the decline of utilization or abandonment of marsh areas (Rosenthal & Hölzel, 2009) and in grasslands on drained bogs (Blankenburg, 2015).

Moreover, the biogeographic history of *D. cespitosa* may have had a long interaction with human activities during the Anthropocene. The plant is included in a list of grasses supposed to have provided textile fibres for clothes made in the prehistoric and protohistoric ages (Mitschke, 2001). Nettings of grass fibres were also found in the shoes and coat of the Iceman 'Ötzi' (Grömer & Schierer, 2005). *Deschampsia cespitosa* was probably among the grasses recognized from epidermal remnants in the Iceman samples (Acs *et al.*, 2005). Clearing of subalpine forest and scrub for high mountain pasturing since Medieval times has favoured grasslands, especially those in which *D. cespitosa* can thrive (Brande *et al.*, 2017).

At present *D. cespitosa* is a frequent component in European seed mixtures offered by many commercial websites (seed traders, gardeners) for various purposes. Some of these mixtures are designed for revegetation of embankments after construction work and in improvement programmes for river or stream banks and shores of lakes and ponds (Frank, 2018). The grass has been used for revegetation trials on ski pistes and flow regulation of mountain streams (Krautzer *et al.*, 2007) and in revegetation programmes in the Alps before more adequate grasses for high alpine regions were available (Wittmann & Rücker, 2012). *Deschampsia* has been used in a German marshland restoration programme (Klötzli, 1980) and in phytoremediation programmes for contaminated soils (Sas-Nowosielska *et al.*, 2008). *Deschampsia* seeds were found in hay transferred for restoration purposes in northern Germany (Rasran *et al.*, 2006), in epizoochorously dispersed seed samples collected from fur and in hairs of large livestock herbivores that were transferred between isolated nature reserves (Couvreur *et al.*, 2004). In Iceland, *D. cespitosa* subsp. *cespitosa* is common in nearly all regions. The species is almost exclusively found close to human settlements. This suggests that human-mediated dispersal could have also played a role in the re-colonization of Iceland. Such a distribution pattern is not present in *D. cespitosa* subsp. *neoalpina*. Affinities of *D. cespitosa* plants of the Murmansk region to those of Iceland and western Scandinavia may result from natural agents (wind, ocean currents), as well as accidental or deliberate human mediated LDD.

TAXONOMIC IMPLICATIONS

Current taxonomy of European *Deschampsia* is based on morphology (Clarke, 1980; Chiapella, 2000) and some regional taxonomic summaries (e.g. Conert, 1987; Amphlett, 2019; McAllister & Amphlett, 2022). A few northern European samples [*D. cespitosa* subspp. *cespitosa* and *neoalpina* and *Deschampsia cespitosa* subsp. *bottnica* (Wahlenb.) Tzvelev] were used by

Chiapella (2007) in a phylogenetic study including related genera. The ITS sequences did not resolve among *D. cespitosa* subspp. *cespitosa* and *bottnica* in a poorly supported clade which was part of a huge polytomy containing several *Deschampsia* spp. from other continents. More recently Peintinger *et al.* (2012) used AFLPs to show substantial divergence of *D. cespitosa* subsp. *rhenana* from *D. cespitosa* subsp. *cespitosa*, whereas *D. cespitosa* subsp. *littoralis* was found in an intermediate position. This pattern remained consistent using a large sample of the entire continent in our present study. Using AFLPs, substantial genetic divergence of *D. cespitosa* subsp. *wibeliana* was also found besides ecological separation from *D. cespitosa* subsp. *cespitosa* by Heydel *et al.* (2017). Therefore, there are good arguments for keeping the subspecies *D. cespitosa* subsp. *cespitosa*, *rhenana*, *littoralis* and *wibeliana*, whereas the status of *D. cespitosa* subsp. *bottnica* needs further investigation. The widespread *D. cespitosa* subsp. *parviflora*, recently again distinguished from *D. cespitosa* subsp. *cespitosa* by features of leaves (colour, scabridness) and spikelet size (McAllister & Amphlett, 2022), is not supported by our genomic analysis. We consider it a forest and shadow variant of the plastic *D. cespitosa* subsp. *cespitosa*. Furthermore, we found that *D. cespitosa* subsp. *neoalpina* is substantially differentiated from all samples of *D. cespitosa* subsp. *cespitosa* even when they co-occur. The strong divergence of Spanish samples of diploid and tetraploid *D. cespitosa* subsp. *cespitosa* may in part result from the sampling design and require some futher attention.

CONCLUSION

Our study provides a first insight into the *D. cespitosa* complex using genomic data on a large scale in Europe. RADseq results showed a high divergence of Iberian populations and a strong separation of pseudoviviparous tetraploids from seminiferous tetraploids (except *D. cespitosa* subsp. *littoralis*) and all diploids of the remains of Europe. The low genetic divergence and in part fuzzy geographical pattern points to a concerted action of several factors: (1) isolation during the LGM and recolonization from various refugial areas; (2) reproductive features such as outcrossing, wind pollination and effective wind dispersal of seeds in an open tundra landscape; and (3) increasing human impact on dispersal as this grass is used for many purposes. The high divergence of the pseudoviviparous tetraploids (*D. cespitosa* subspp. *neoalpina* and *rhenana*) from the seminiferous tetraploids (except *D. cespitosa* subsp. *littoralis*) and all (in part co-occurring) diploids indicates a strong isolation of those presumably ancient tetraploids.

Other tetraploid *D. cespitosa* are always nested in diploid *D. cespitosa* suggesting multiple autopolyploid origins. Plastid SNPs show weak genetic structure and more haplotypes existing in central Europe than in other regions, probably due to colonization by diverse maternal lineages.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY

RAD and whole genome sequence data for this study are available in the NCBI Short Reads Archive, and they can be accessed with the BioProject number PRJNA887944 for RADseq data and PRJNA886965 for the whole genome sequence data.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article on the publisher's website.

Table S1. *Deschampsia* sampling details for each locality in this study.

Figure S1. ΔK statistics (Evanno *et al.*, 2005) to identify the optimal number of gene pools in different datasets: A, dataset 1, B, smalldataset 1, C, dataset 2, D, dataset 3, E, dataset 4.

Figure S2. Clustering patterns of 147 accessions of *Deschampsia* (smaller dataset from dataset 1). A, heatmap obtained with covariance matrix results from PCAngsd. Locality information is at the bottom, the country names are on the left and the species names are on top. B, PCA results based on 1 479 184 polymorphic sites from PCAngsd results. Species (and subspecies) and genetic groups (K = 6) are represented in different colours. C, genetic structure and admixture based on 20 093 unlinked polymorphic sites from ANGSD. Admixture diagrams are shown for K = 2–6. Locality information is at the bottom and species names are on top. Abbreviations: *ces*: subsp. *cespitosa*; *par*: subsp. *parviflora*; *rhe*: subsp. *rhenana*; *lit*: subsp. *littoralis*; *alp*: subsp. *neoalpina*; *arg*: *Deschampsia argentea*; Iberian: Spanish *D. cespitosa* subsp. *cespitosa* and the Macaronesian island endemic *D. argentea*; NW and central Eur: north-western and central Europe; NE Eur: north-eastern Europe; AUT: Austria; DEU: Germany; ISL: Iceland; CHE: Switzerland; ROM: Romania; RUS: Russian Federation; ESP: Spain; PRT: Portugal.

Figure S3. Clustering patterns of 326 diploid accessions from dataset 1 (referred detaset 3). A, genetic structure and admixture map from ANGSD. Admixture diagrams are shown for K = 3. B, PCA results of 326 accessions based on 1 028 038 polymorphic sites from PCAngsd results. Genetic groups (K = 3) are represented in different colours. Abbreviation: *ces*: subsp. *cespitosa*; *arg*: *Deschampsia argentea*; Iberian: Spanish *D. cespitosa* subsp. *cespitosa* and the Macaronesian island endemic *D. argentea*; NW central Europe: north-western and central Europe; NE Europe: north-eastern Europe.

Figure S4. Violin plot showing pairwise F_{ST} between localities used for comparing locality133 of the Murmansk region with localities in six genetic groups. Abbreviations: ICEWS: Iceland and western Scandinavia; RUS: eastern Europe (western Russia including three trans-Ural localities); WE: western central Europe; ECE: eastern central Europe extending to the north in eastern Scandinavia; CEalp: central Europe with the Alps; Iberian: Spanish *D. cespitosa* subsp. *cespitosa* and the Macaronesian island endemic *D. argentea*.