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Genetic structure and evolution of diploid *Cochlearia* in Iceland

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In northern European *Cochlearia* (Brassicaceae), considerable chromosome variation has taken place without corresponding morphological differentiation, resulting in an intricate species complex including two base chromosome numbers and several ploidies. Here, we investigate the situation in Iceland. The distribution, genetic structure, taxonomy and origin of the two *Cochlearia* cytotypes ($2n = 12$ and $2n = 14$) present in Iceland are discussed. Chromosome counts indicate that both cytotypes occur along the coast, but $2n = 12$ populations dominate (eight $2n = 12$ vs. two $2n = 14$ among the investigated populations), whereas $2n = 14$ was reported for the two inland alpine populations investigated here. RADseq data support geographically structured genetic variation along the Icelandic coast and environmentally structured genetic differentiation between coastal and alpine populations. The alpine populations show genetic and morphological affiliation with *C. groenlandica* ($2n = 14$), which is widely distributed in the Arctic, but more comprehensive sampling is needed to draw conclusions concerning the taxonomic status of the Icelandic coastal plants. To uncover the origin of and phylogenomic relationships between the two chromosome variants, comparative whole-genome sequencing should be performed.

ADDITIONAL KEYWORDS: alpine – Brassicaceae – chromosome counts – coastal plant – dysploidy – morphology – RADseq.

INTRODUCTION

Cochlearia L. (Brassicaceae) is distributed in coastal and inland (alpine) habitats throughout Europe and the circumpolar region, with 15–16 accepted species according to BrassiBase (Kiefer *et al.*, 2014) and Plants of the World Online (POWO, 2021). It is known as a notoriously difficult group when it comes to taxonomic delineation. The opening quote of Hooker (1861), on his observation of morphological traits > 150 years ago, is still applicable today: '[*Cochlearia*] has always proved to me to be one of the most intractable boreal genera […] habit, pods and leaves afford the characters hitherto made use of; and all are equally fallacious, as far as affording permanents distinctions'. Hultén (1971) followed up by stating that *C. officinalis* L. *s.l.* is 'a very complicated complex treated differently by practically all students of the group', and later studies have confirmed that much of the morphological

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variation that has been given taxonomic weight is of environmental origin (Elkington, 1984; Nordal & Stabbetorp, 1990).

Cochlearia split off from its sister genus *Ionopsidium* Rchb. *c.* 13.80–9.25 Mya in the mid-Miocene (Koch, 2012; Hohmann *et al.*, 2015; Wolf *et al.*, 2021), but divergence within the genus did not start until the mid or late Pleistocene, *c.* 0.66 Mya (Koch, 2012; Wolf *et al.*, 2021). Compared to *Ionopsidium*, *Cochlearia* is regarded as a cold-tolerant genus (Koch, 2012), and metabolic responses to cold have been found in different taxa of the genus inhabiting alpine environments (Wolf *et al.*, 2021).

In this young group, considerable chromosome evolution has been encountered without corresponding morphological differentiation (Nordal & Stabbetorp, 1990; Koch, Hurka & Mummenhoff, 1996). Two base chromosome numbers, $n = 6$ and $n = 7$, have evolved into two phylogenetic lineages. Subsequently, auto- and allopolyploidy within (and possibly between) these lineages have resulted in large ploidy variation, ranging from diploidy to decaploidy (e.g. Saunte, 1955; Gill, 1973; Nordal *et al.*, 1986; Koch *et al.*, 1996; Koch, Mummenhoff & Hurka, 1999; Cieślak, Cieślak & Ronikier, 2021). Hybrids between *Cochlearia* taxa are reported from nature (e.g. Fearn, 1977; Nordal & Laane, 1996; Pegtel, 1999), and crossing experiments across ploidies resulted in fully or partially fertile hybrids (e.g. Crane & Gairdner, 1923; Saunte, 1955; Gill, 1971; Kenich, 2020). Based on cytological studies after hybridization of $2n = 14$ plants from Iceland, Greenland and Arctic North America (*C. groenlandica* L.) with $2n = 12$ plants from south-western and central Europe [*C. pyrenaica* DC. and *C. aestuaria* (J.Lloyd) Heywood], Gill (1971, 1973) suggested that the $n = 7$ karyotype was derived from $n = 6$ by primary tetrasomy (doubling of one chromosome pair).

Presently, diploids with $2n = 12$ are distributed from south-western and central Europe through Britain and Ireland Isles to Iceland (Nordal, 1988), whereas diploids with $2n = 14$ are mainly high-Arctic and widely distributed in all major Arctic regions from Alaska to the Russian Far East (Elven, 2011). Iceland is the only area where plants with both chromosome numbers co-occur (Löve & Löve, 1956, 1976; Löve, 1975; Gill, 1971; Nordal & Laane, 1990; Koch, Huthmann & Hurka, 1998; Wolf *et al.*, 2021). Chromosome numbers have, so far, only been obtained from a limited number of Icelandic localities (Supporting Information, Table S1). Two ecologically differentiated *Cochlearia* forms exist in Iceland. A larger 'coastal' form grows on beach cliffs along the coast, whereas a dwarfed 'alpine' form is occasionally found in late snow beds on inland mountains at 700–1000 m a.s.l. (Kristinsson, 2010; Kristinsson, Hlíðberg & Þórhallsdóttir, 2018; Wasowicz, 2020). Both diploid chromosome numbers are reported for coastal plants, with $2n = 12$ plants reported from the southern coast of Iceland (Gill, 1971; Löve, 1975; Nordal & Laane, 1990) and $2n = 14$ plants mainly from the northern and western coast of Iceland (Saunte, 1955; Gill, 1971; Nordal & Laane, 1990). So far, only 2n = 14 has been reported for the alpine plants (Nordal & Laane, 1990).

For a long time, there has been confusion on how to name the Icelandic *Cochlearia*; this has greatly varied depending on authors (Supporting Information, Table S2), partly reflecting the split between coastal and alpine plants and partly the difference in chromosome number. Some authors (e.g. Stefánsson, 1924, 1948) used the variety level to separate alpine plants [*C. officinalis* var. *groenlandica* (L.) Gelert] from coastal plants [*C. officinalis* vars. *oblongifolia* (DC.) Gelert and *arctica* (DC.) Gelert], whereas others (e.g. Pobedimova, 1970) recognized alpine plants (*C. groenlandica*) and coastal plants (*C. islandica* Pobed. and *C. anglica* L.) as different species. Based on different chromosome numbers, Löve & Löve (1976) and Löve (1983) referred Icelandic plants not only to different species but to different genera, *C. pyrenaica* ($2n = 12$) and *Cochleariopsis groenlandica* (L.) Á.Löve & D.Löve subsp. *islandica* (Pobed.) Á.Löve ($2n = 14$). The most recent taxonomic annotation by the Icelandic Institute of Natural History concludes that the name of the alpine type should be *C. groenlandica*, whereas the coastal type, regardless of chromosome number variation, should be *C. islandica* (Wasowicz, 2020).

Few molecular studies (e.g. Koch *et al.*, 1996, 1998) have been conducted including Icelandic *Cochlearia* plants, and it is so far unclear whether they belong to the same genetic cluster, or whether they represent more genetic clusters corresponding to chromosome number and/or habitat. Recently, Wolf *et al.* (2021) included a few plants from Iceland in phylogenetic analyses based on plastid and nuclear genome sequences. Their analyses divided *Cochlearia* into two main gene pools, separating European samples from Arctic samples, with varying degrees of admixture in the areas between, especially in Iceland and western Canada.

The aim of this study is to investigate further the genetic structure and evolution of the Icelandic *Cochlearia* populations. Single-nucleotide polymorphisms (SNPs) obtained from restriction site-associated DNA sequencing (RADseq) are used to detect genetic structure. The genetic groups are compared to variation in chromosome numbers, spatial distribution, environmental conditions and leaf and floral traits to investigate which mechanisms best explain the genetic structure. We specifically address whether plants with different chromosome numbers ($2n = 12/14$) and/or ecology (coastal/alpine) constitute genetic clusters.

We also discuss the evolutionary relationship of Icelandic populations to diploid *Cochlearia* from other geographical regions and possible taxonomic/nomenclatorial solutions.

MATERIAL AND METHODS

Plant material

Plant material was obtained from 15 Icelandic populations (abbreviation of locality names is used throughout the paper according to Table 1). Thirteen of the sampled populations were located close to the sea and considered 'coastal', and two populations sampled from inland (920–1030 m a.s.l.) localities were considered 'alpine'. Sampling localities were chosen on the basis of previous publication of chromosome numbers (Saunte, 1955; Gill, 1971; Löve, 1975; Nordal & Laane, 1990; Koch *et al.*, 1998; Supporting Information, Table S1) or from locality records obtained from a database at the Icelandic Institute of Natural History. We intended to obtain a representative collection of populations along the coast, but were specifically interested in including populations along the southern coast from where $2n = 12$ populations have previously been reported (Gill, 1971; Löve, 1975; Nordal & Laane, 1990). We succeeded only in collecting two alpine populations, but these two populations represent the two main distributional groups in the high mountains of northern Iceland, an eastern group and a more central group, separated by the Mid-Iceland Belt (MIB) comprising a large area of active volcanoes, glaciers and lava fields (GBIF Secretariat, 2021). From most field localities, (1) healthy green leaves were harvested and dried instantly on silica gel for subsequent DNA extraction, (2) leaves were pressed and flowers placed in 70% ethanol for morphometric studies, (3) whole inflorescences containing flower buds were treated with two shifts of freshly prepared Carnoy's fixative I (3:1 ethanol:glacial acetic acid), kept in 70% ethanol at –20 °C for long-term storage and later used for cytological studies and (4) seeds were collected when present. Seeds were later germinated and plants grown under controlled conditions in growth chambers at the University of Oslo (18 h light at 18 °C; 6 h dark at 10 °C) to obtain additional material for morphometric and cytological analyses. Reference material of diploid *Cochlearia* taxa from Svalbard (*C. groenlandica*) and south-western Europe (*C. aestuaria*, *C. pyrenaica*) were included in the molecular analyses (Supporting Information, Table S3). Herbarium vouchers of field-collected specimens are deposited in the herbarium at the Natural History Museum, University of Oslo (O) or the University of Oviedo (FCO) (Table 1; Supporting Information, Table S3).

Chromosome sampling

Chromosome spreads from flower buds were prepared for 12 of the Icelandic *Cochlearia* populations (Table 1), for one sample per population (except that four samples were counted from population BAE). Inflorescences were fixed in freshly prepared fixative (ethanol:acetic acid, 3:1) at 4 °C overnight, transferred into 70% ethanol and stored at –20 °C until use. Chromosome spreads were prepared according to the protocol published by Mandáková *et al.* (2016). Chromosome numbers were recorded from microscope photographs after counterstaining with DAPI (4´,6-diamidino-2-phenylindole; 2 µg/mL) in Vectashield. For chromosome counts conduct at the Masaryk University, chromosomes were photographed using an Olympus BX-61 epifluorescence microscope equipped with a Zeiss CoolCube camera. Greyscale images were processed using Adobe Photoshop CS2 software. Chromosome counts for three populations (STR, HAF and SUR) were made at the University of Oslo and examined using a Zeiss Axioplan Imaging2 epifluorescence microscope equipped with Nomarski optics and the Zeiss AxioVision 4.8 software.

RADseq library preparation

We extracted genomic DNA from *c*. 30 mg dried leaf sample using the E.Z.N.A. SP plant DNA kit (Omega bio-tek, Norcross, GA, USA) following the protocol for dry samples with minor modifications. The dried samples were crushed with two 3-mm tungsten carbide beads (Qiagen, Venlo, Netherlands) for 2 min at 20 Hz in a TissueLyser II, Retsch MMo1 (Retsch, Castleford, UK). Most samples were eluted in 100 µL of elution buffer. Samples with considerably less starting material than 30 mg were first eluted in 50 µL of elution buffer, and then once again using the first eluate to increase the final concentration of the extracted DNA. DNA extractions were cleaned with NucleoSpin gDNA Clean-up (Macherey-Nagel, Düren, Germany). Quantification and quality check of the extracted DNA were performed using NanoDrop ND-1000 V3.10 Spectrophotometer (Thermo Scientific, Waltham, MA, USA) and Qubit dsDNA BR assay kit (Life Technologies, Carlsbad, CA, USA) with a Qubit fluorometer (Life Technologies).

We prepared a RADseq library using single digest, double barcoding and size selection with magnetic beads according to a protocol adapted from Baird *et al.* (2008) and Paun *et al.* (2016), with modifications as in Brandrud *et al.* (2017). The library included 79 samples (and three replicates) of which 73 *Cochlearia* samples from Iceland and Svalbard were included in the present study (Table 1; Supporting Information, Table S3). For each sample, 250 ng of DNA was

Table 1. Locality, collection and voucher information for 15 Icelandic *Cochlearia* populations, with number of samples used for RADseq analyses (including replicates = repl.), morphometrics (field collected + cultivated) and chromosome counts (obtained chromosome number in brackets). Vouchers are kept in the herbarium at the Natural History Museum, University of Oslo (O) and Icelandic Institute of Natural History, Division Akureyri (AMNH). Population abbreviations are used throughout the paper. Locality information for reference populations of diploid *Cochlearia* from south-western Europe (*C. aestuaria* and *C. pyrenaica*) and Svalbard (*C. groenlandica*) is given in the Supporting Information, Table S3

Population name/ abbreviation	Locality	Longitude, latitude	Collection date (collectors*)	Collection number (herbarium voucher)	Number of samples analysed		
					RADseq	morpho-metrics	chromo-some counts
Southern Iceland							
DYR	Dyrhólarey, Skaftafellssýsla	−19.13028, 63.40192	12.08.2014 (AKB, IN, LNO)	LO.14-2 (O)	5	2 + 4	1 ($2n$ = 12)
HJO	Hjörleifshöfði, Vestur-Skaftafellssýsla	−18.76528, 63.42031	12.08.2014 (AKB, IN, LNO)	LO.14-3 (O)	4		
ING	Ingólfshöfði Austur-Skaftafellssýsla	−16.63778, 63.80400	13.08.2014 (AKB, IN, LNO)	LO.14-4 (O)	5	3 + 4	1 ($2n$ = 12)
SUR	Surtsey, Vestmanna-eyjar	−20.59340, 63.30010	2014 (PW)	06160714 (O, AMNH)	3		1 ($2n$ = 12)
Eastern Iceland							
HVA	Hvalnes, Austur-Skaftafellssýsla	−14.54456, 64.40328	13.08.2014 (AKB, IN, LNO)	LO.14-5 (O)	5	5 + 4	1 ($2n$ = 12)
DJU	Djúpivogur, Suður-Múlasýsla	−14.27983, 64.65667	13.08.2014 (AKB, IN, LNO)	LO.14-6 (O)	5	5 + 3	1 ($2n$ = 12)
STR	Strandhöfn, Norður-Múlasýsla	−14.64700, 65.90400	14.08.2014 (AKB, IN, LNO)	LO.14-8 (O)	5 (+ 1 repl.)	4 + 1	1 ($2n$ = 12)
HFN	Hafnarhólmur, Norður-Múlasýsla	−13.75475, 65.54207	2014 (PW)	02310714	3		
Western and northern Iceland							
BAE	Bær á Höfðaströnd, Skagafjarðarsýsla	−19.43600, 65.93000	15.08.2014 (AKB, IN, LNO)	LO.14-10 (O)	5	4 + 4	4 ($2n$ = 12)
LAT	Látrabjarg, Vestur-Barðastrandarsýsla	−24.53071, 65.50150	2014 (PW)	01090814	3		
OLA	Ólafsvíkurenni Snæfells- og Hnappadalssýsla	−23.76589, 64.90272	16.08.2014 (AKB, IN, LNO)	LO.14-11 (O)	4 (+ 1 repl.)	5 + 4	1 ($2n$ = 12)
HAF	Hafnir, Gullbringusýsla	−22.68669, 63.93592	17.08.2014 (AKB, IN, LNO)	LO.14-12 (O)	5	4 + 4	1 ($2n$ = 14)
STO	Stokkseyri, Árnessýsla	−21.07519, 63.83858	11.08.2014 (AKB, IN, LNO)	LO.14-1 (O)	5 (+ 1 repl.)	4 + 4	1 ($2n$ = 14)
Alpine Iceland							
EIR	Eiríksstaðir, Norður-Múlasýsla	−15.47175, 65.14344	14.08.2014 (AKB, IN, LNO)	LO.14-7 (O)	4	4 + 3	1 ($2n$ = 14)
GIL	Gilsbakki, Skagafjarðarsýsla	−18.96622, 65.37028	15.08.2014 (AKB, IN, LNO)	LO.14-9 (O)	4	5 + 4	1 ($2n$ = 14)

*Collectors: AKB—A. K. Brysting, IN—I. Nordal, LNO—L. N. Olsen, PW—P. Wasowicz.

digested with 15 U *Pst*I-HF (NEB, New England Biolabs, Ipswich, MA, USA) at 37 °C for about 2 h. Since *Pst*I-HF cannot be heat activated, the samples were cleaned with SPRIselect Reagent Kit (Beckman Coulter, Indianopolis, IN, USA) with no size selection. After addition of P5 adapters, we pooled the samples into seven sub-libraries, which were kept separate throughout the procedure. The DNA was sheared by sonication, using nine cycles (30 s 'on' and 30 s 'off') at 2 °C on a Bioruptor Plus (Diagenode, Denville, NJ, USA), to obtain an optimal size of 300–600 bp. The samples were cleaned using MinElute Reaction

Clean-up Kit (Qiagen) followed by left (0.7×) and right (0.55×) side size selection with the SPRI select Reagent Kit. After ligation of P7 adapters, similar cleaning and size selection (this time only on the left side; 0.65×) were done, both before and after polymerase chain reaction (PCR) amplification with Phusion Master Mix (NEB). The concentration of each sub-library was measured using quantitative PCR (qPCR) assay (KAPA Library Quantification Kits catalogue number KK4824, KAPA Biosystems, MA, USA) using a qPCR cycler (Lightcycler 96, Roche, Basel, Switzerland) and ensuring that equal amounts of each sub-library were included in the final RAD_{seq} library. The library was sequenced using paired-end sequencing (125 bp) in one Illumina HiSeq2500 lane at the Norwegian Sequencing Centre, Oslo, Norway (http://www.sequencing.uio.no/).

Processing of RADseq reads

Raw Illumina reads were processed with STACKS v.1.29 (Catchen *et al.*, 2011, 2013), using the Norwegian national infrastructure for computational sciences (Norwegian Metacentre for High Performance Computing, NOTUR; now replaced by UNINETT Sigma2). To demultiplex samples and remove low quality data, process_radtags was run with the following settings: *Pst*I as restriction enzyme, removal of any read with an uncalled base, discarding reads with low quality scores, and rescuing barcodes and RADtags. The retained read numbers per sample after demultiplexing ranged from 1 186 173 to 4 753 514. Denovo.map.pl was used to execute ustacks, cstacks and sstacks with the forward reads. Different values for m (minimum number of identical raw reads required to create a stack), M (number of mismatches allowed between loci when processing a single individual) and N (number of mismatches allowed between loci when building the catalogue) were tested to find the combination that maximized the number of reliable loci. The settings used in the end were $m = 2$, $M = 2$ and $N = 1$.

A high number of unique loci in the processed RADseq library raised suspicion about contamination. The sequence reads were therefore aligned to the *Arabidopsis thaliana* (L.) Heynh. reference genome using the National Centre for Biotechnology Information (NCBI). Less than 30% of the tested reads aligned more than once to the reference genome, *c.* 10% aligned once and the rest did not align at all. Blasting of the data in the NCBI Nucleotide collection revealed that *c.* 77% of the reads were indeed from bacteria and fungi; some of them known endophytes of *A. thaliana*. However, the probability of a read stemming from bacteria or fungi decreased steeply with increasing number of individuals for which a read was scored, and almost all reads scored in at least ten individuals had Brassicaceae-like BLAST hits. To remove bacterial reads from the output files used for downstream data analysis, we implemented strict filter settings when running populations in STACKS to link individuals to their respective population, retaining only loci that were present in at least 80% of the individuals in a population, and in at least 70% of the populations. Filtered reads were further blasted to check for remaining bacterial DNA, and no contamination was observed. In the final output files (structure and phylip), only one SNP per locus was retained (i.e. the first SNP in each locus) to minimize as much as possible linkage of markers. As replicated samples clustered together in initial data analyses, only one per accession was included in the final analyses.

Forward reads of ten diploid individuals from south-western Europe (*C. aestuaria* and *C. pyrenaica*; Supporting Information, Table S3) from a RADseq library produced as part of another study (Brandrud *et al.*, 2017) were processed and analysed with the 73 *Cochlearia* samples from Iceland and Svalbard, resulting in a total of 83 samples.

Downstream analyses of SNPs from RADseq

We investigated population structure for the whole dataset (83 individuals) with STRUCTURE v.2.3.3 (Pritchard, Stephens & Donnelly, 2000) using the admixture model and correlated frequencies (Falush, Stephens & Pritchard, 2007). The dataset was run with $K = 1$–20, ten runs for each K, 1 000 000 iterations and burn-in of 100 000. We summarized the results in STRUCTURE HARVESTER web v.0.9.94 (Earl & vonHoldt, 2012) and CLUMPAK beta v. (Kopelman *et al.*, 2015), producing likelihood graphs and deltaK graphs (Evanno, Regnaut & Goudet, 2005). The optimal number of groups converged to the same solution for all replicate runs (confirmed by inspecting the plots), and was visualized using DISTRUCT v.1.1 (Rosenberg, 2004) and as pie charts on a map of Iceland using QGIS v.2.18.17 (QGIS.org, 2020). We used the phylip file for the whole dataset (83 individuals) to produce a phylogenetic network in SPLITSTREE v.4.11.3 (Huson & Bryant, 2006). Splits were created from Jukes Cantor distances and visualized as a neighbour net with each end node representing an individual. Principal component analysis (PCA) was performed on *Cochlearia* individuals from Iceland and Svalbard (73 individuals), in R (R Core Team, 2020) and RStudio (RStudio Team, 2020) with the R package adegenet v.1.4.2 (Jombart, 2008; Jombart & Ahmed, 2011) using allele frequencies centred to mean zero and scaled, missing values treated as zero and Euclidean distances. A neighbours list was created from the geographical coordinates with the function tri2nb with the R package spdep v.1.1.2 (Bivand, Pebesma &

Gomez-Rubio, 2013). Monmonier's algorithm was then used to look for potential genetic boundaries among the Icelandic populations (65 individuals) using the R package adegenet v.2.1.1 (Jombart, 2008; Jombart & Ahmed, 2011). Genetic boundaries were detected using the structure file and visualized on the map of Iceland with the STRUCTURE pie charts.

To estimate genetic differentiation among populations and among higher-level groups, we ran analyses of molecular variance (AMOVA) in ARLEQUIN v.3.5.2.2 (Excoffier & Lischer, 2010) after the structure file was converted to the appropriate format in PGDSpider v.2.0.8.2 (Lischer & Excoffier, 2012). Analyses using chromosome number and geography as higher-level groups were run for the whole dataset (83 individuals), and for a dataset including only the Icelandic populations (65 individuals). Private alleles found between groups defined by geography and chromosome number were calculated by applying the gl.filter.pa function in the R package dartR v.1.8.3 (Gruber *et al.*, 2018) for the whole dataset. A heatmap was created from the allele frequencies of all populations with the gl.grm function in dartR.

To investigate the genetic data on the Icelandic populations in the context of geographical and environmental factors, several tests were performed. An isolation by distance test was performed between pairs of populations with a mantel.randtest (999 permutations) in the R package *ade4* v.1.7.13 (Dray & Dufour, 2007) and displayed as a plot. To understand better the observed pattern, the fine-scale spatial structures were assessed by testing if the average observed relatedness predicted by a local polynomial fitting (LOESS) differed from the null model (with 95% confidence bounds), with 999 permutations. This was performed calculating Yang's genetic relatedness (Yang *et al.*, 2010) between pairs of individuals and applying the Lplot function (https://github.com/rojaff/Lplot) used in Carvalho *et al.* (2019). The analysis was run both with and without the two alpine populations, which did not change the resulting neighbourhood size much. Further, bioclimatic variables likely to describe the difference between the ecologies inhabited by *Cochlearia* were downloaded from Worldclim Global climatic data (Hijmans *et al.*, 2005): annual mean temperature, annual precipitation and elevation. These variables were correlated with ecotype (scoring coastal as 0 and alpine as 1) in a correlation matrix by applying rquery.cormat in the R package corrplot v.0.84 (Wei & Simko, 2017). As both annual mean temperature and elevation had a high absolute value for the correlation with ecotype, these variables were further tested with a mantel.randtest (999 permutations) and displayed in an isolation by environment plot where annual mean temperature difference was displayed by the position of the points and elevation by colour.

Morphometric analyses

For most (11) Icelandic populations, we measured leaf traits on pressed material from both field-collected plants and plants cultivated in controlled conditions. Populations HFN, HJO, LAT and SUR were only available as silica-dried material and were not included in the morphometric analyses. When possible, we measured five leaves from five samples per population. The leaf length (L), width (W), leaf ratio (W/L) and leaf base angle, previously recognized by Nordal & Laane (1990) as informative, were measured/calculated (Supporting Information, Fig. S1).

For five populations, we measured flower traits on field-collected and cultivated plants. For five populations (DYR, GIL, HAF, STR, STO), only cultivated material was available, and for one population (OLA), only field-collected material was available. When possible, we measured three flowers from four samples per population. The flower traits petal length (PL), petal width (PW), sepal length and petal ratio (PW/PL) were measured and/or calculated.

Tests for critical requirements of homoscedasticity (Levene, 1960) and normality (Shapiro & Wilk, 1965) for parametric analyses showed that these requirements were not fulfilled. Subsequently, the non-parametric Kruskal–Wallis test (Kruskal & Wallis, 1952) was performed to test for significant population differences, and further Dunn's post hoc test (Dunn, 1964) with Bonferroni corrected P values was used to test which populations were significantly different. Summary statistics and statistical analyses were performed using the R packages dplyr v.0.7.6 (Wickham *et al.*, 2019) and dunn.test v.1.3.5 (Dinno, 2017). Boxplots of all measured and calculated traits were made in Microsoft Excel 2016. PAST 4.03 (Hammer, Harper & Ryan, 2001) was used to run principal coordinate analysis (PCO) on measured leaf and flower traits, respectively, using Gower similarity index (Gower, 1971).

RESULTS

Chromosome counting

Four populations, among them the two alpine populations, had a chromosome number of $2n = 14$ (EIR, GIL, HAF, STO) and eight populations (BAE, DJU, DYR, HVA, ING, OLA, STR, SUR) had a chromosome number of $2n = 12$ (Fig. 1, Table 1; Supporting Information, Fig. S2). All four individuals from population BAE had a chromosome number of $2n = 12$ (Suppporting Information, Fig. S3). Chromosome numbers were not obtained for the remaining three populations (HFN, HJO, LAT).

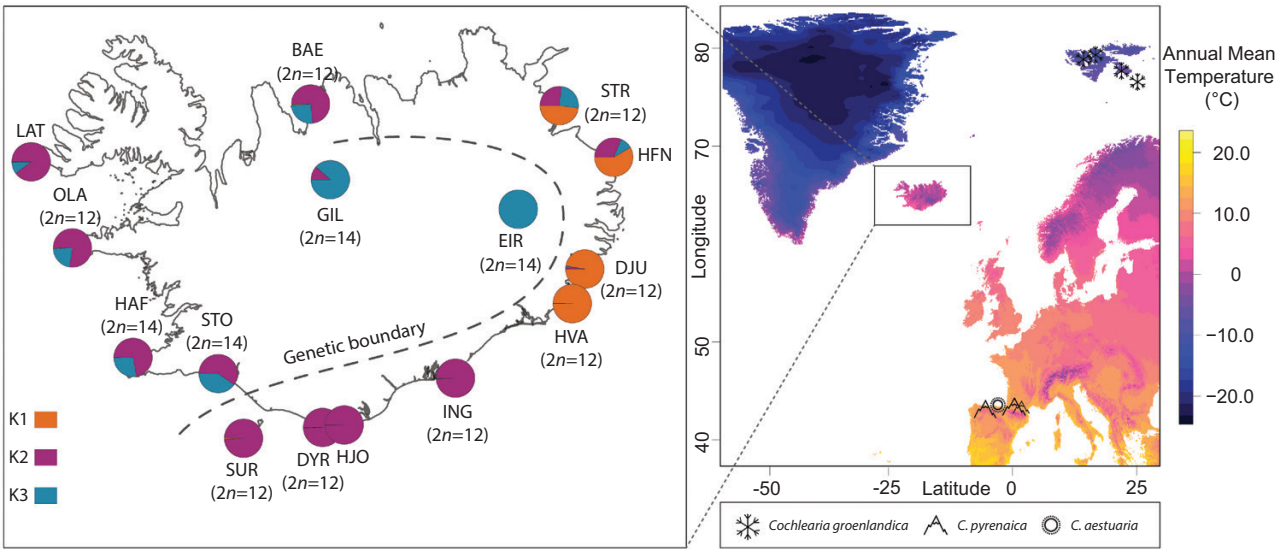

Figure 1. Map of the North Atlantic region with annual mean temperature indicated and figure section showing Iceland with the 15 sampled *Cochlearia* populations. Overall assignment of Icelandic populations to three STRUCTURE groups (K1–K3), based on 1500 RADseq-derived SNPs, are visualized by pie charts. The structure groups are based on $K = 4$, where three groups correspond to Icelandic populations (including also *C. groenlandica* from Svalbard). The last group constitutes the south-western European *C. pyrenaica* and *C. aestuaria* populations (Supporting Information, Fig. S4). Chromosome counts obtained in this study are indicated in brackets under the population abbreviation. The genetic boundary detected by Monmonier's algorithm is indicated with a dotted line. For population abbreviations and further locality information, see Table 1. Annual mean temperature was downloaded from Worldclim Global climatic data (Hijmans *et al.*, 2005). The map layer of Iceland was extracted from GADM version 1.0.

RADseq analyses

From *c.* 349 million paired-end reads obtained from RADseq, *c.* 196 million forward reads were retained after demultiplexing and cleaning. After *de novo* catalogue building and SNP calling, we retained *c.* 12 000 RAD loci present in at least 80% of the 83 individuals. The final structure and phylip files used for downstream data analyses contained 1500 SNPs present in at least 80% of the individuals in a population, and in at least 70% of the populations.

Population structure and genetic boundaries

From the STRUCTURE analysis, $K = 3$ had the highest and $K = 4$ the second highest delta K value, but as $K = 4$ had a higher likelihood value, both were visualized using DISTRUCT (Supporting Information, Fig. S4). When three genetic clusters were selected ($K = 3$; Supporting Information, Fig. S4A), the Icelandic populations were assigned to two of these clusters (purple and turquoise), and the populations from south-western Europe were assigned to the third cluster (blue). Coastal populations from southern Iceland (DYR, HJO, ING, SUR) assigned to the purple clustered with two of the southernmost populations from eastern Iceland (DJU and HVA). The alpine $2n = 14$ populations from Iceland (EIR and GIL) assigned to the turquoise clustered with populations from Svalbard (HOP, TJU, LOM, FLA), except for one admixed sample from GIL. The remaining populations (coastal) showed varying degree of admixture. When four genetic clusters were selected ($K = 4$, Fig. 1; Supporting Information, Fig. S4B), the non-admixed purple cluster were split into southern Iceland (purple) and eastern Iceland (orange). The two northernmost populations from eastern Iceland (HFN and STR) were now admixed between the eastern (orange), the alpine/Svalbard (turquoise) and the southern (purple) clusters. The other populations kept a similar admixed affiliation to that which they had with $K = 3$.

The neighbour-joining network grouped all individuals (except the outlier individual in the alpine GIL population) according to populations (Fig. 2), displaying the same geographical structure as found in the STRUCTURE analysis with clear splits separating south-western European populations from the Icelandic and Svalbard populations, and the Svalbard and alpine Icelandic populations from the coastal Icelandic populations. A minor diagonal split further divided eastern Iceland from southern and northern + western Iceland.

The variation explained along the first principal axis (9.1%) of the PCA plot corresponded to one of the larger splits in the neighbour net, separating the alpine

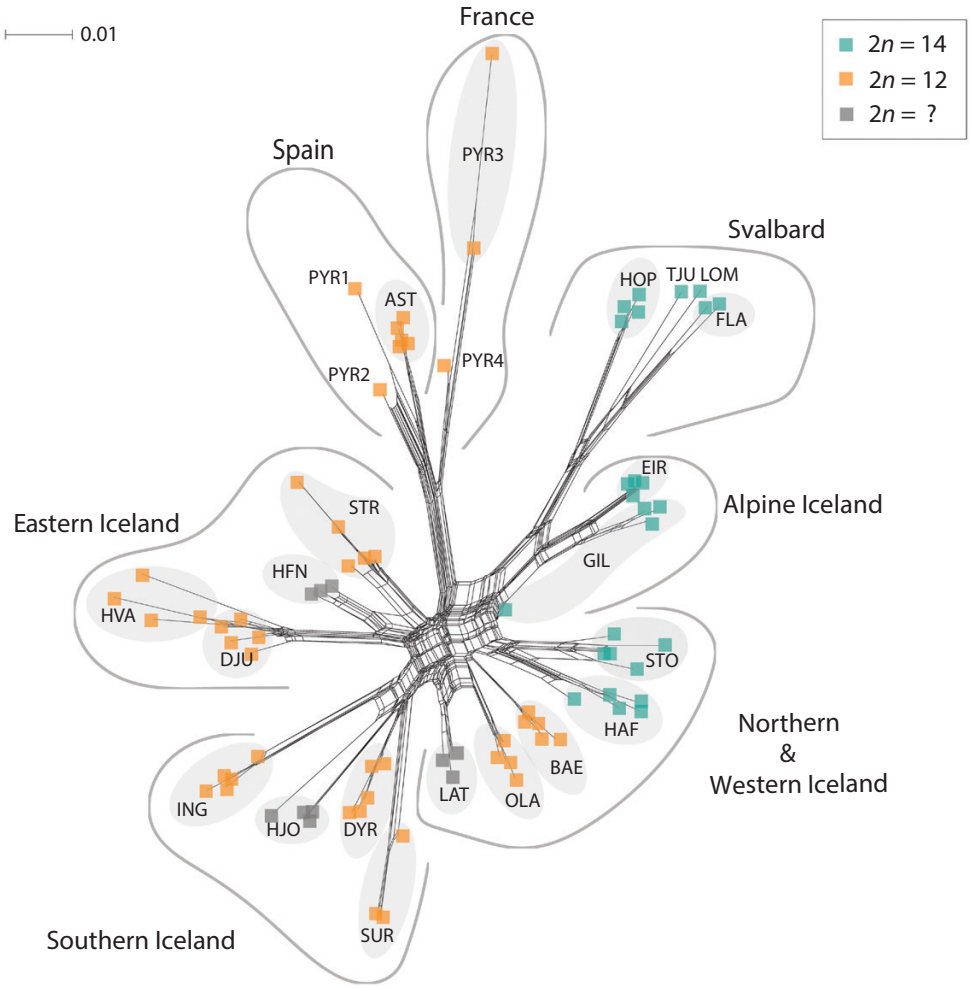

Figure 2. Neighbour net, based on 1500 RADseq-derived SNPs, for 15 Icelandic *Cochlearia* populations and diploid *Cochlearia* from Svalbard (*C. groenlandica*) and south-western Europe (*C. aestuaria* and *C. pyrenaica*). Colours indicate chromosome numbers (orange: $2n = 12$; green: $2n = 14$; grey: chromosome number not available). Approximate geographical areas are indicated for the Icelandic populations: southern Iceland, eastern Iceland, northern and western Iceland and alpine Iceland. For population abbreviations and further locality information, see Table 1 and Supporting Information, Table S3.

Icelandic (except for the outlier sample from GIL) and Svalbard populations from the remaining populations (Fig. 3). PC2 (7.2%) and PC3 (5.1%) showed further subtle structure among the Icelandic material. PC2 mainly separated eastern Iceland from the remaining Icelandic populations (Fig. 3), corresponding to the split in the neighbour net, whereas the southern Icelandic populations (especially DYR) was separated along PC3 (Supporting Information, Fig. S5). The alpine Icelandic populations were separated from the Svalbard populations along PC3. Among the coastal Icelandic populations, the northernmost populations from eastern Iceland (HFN and STR) had an intermediary position, corresponding to the admixture patterns observed in the STRUCTURE analysis. Genetic boundaries were detected that separated the alpine Icelandic populations from populations from eastern and southern Iceland, but no such boundaries were found towards the northern + western Iceland populations (Fig. 1).

Genetic differentiation and private alleles

The AMOVA performed on the whole dataset (Table 2) showed that whereas most of the variance was found among populations within groups, more variance was found among geographical groups (29.84%) than groups defined by chromosome number (13.14%). The same pattern was found when the AMOVA was performed only on the Icelandic populations, with 23.99% of

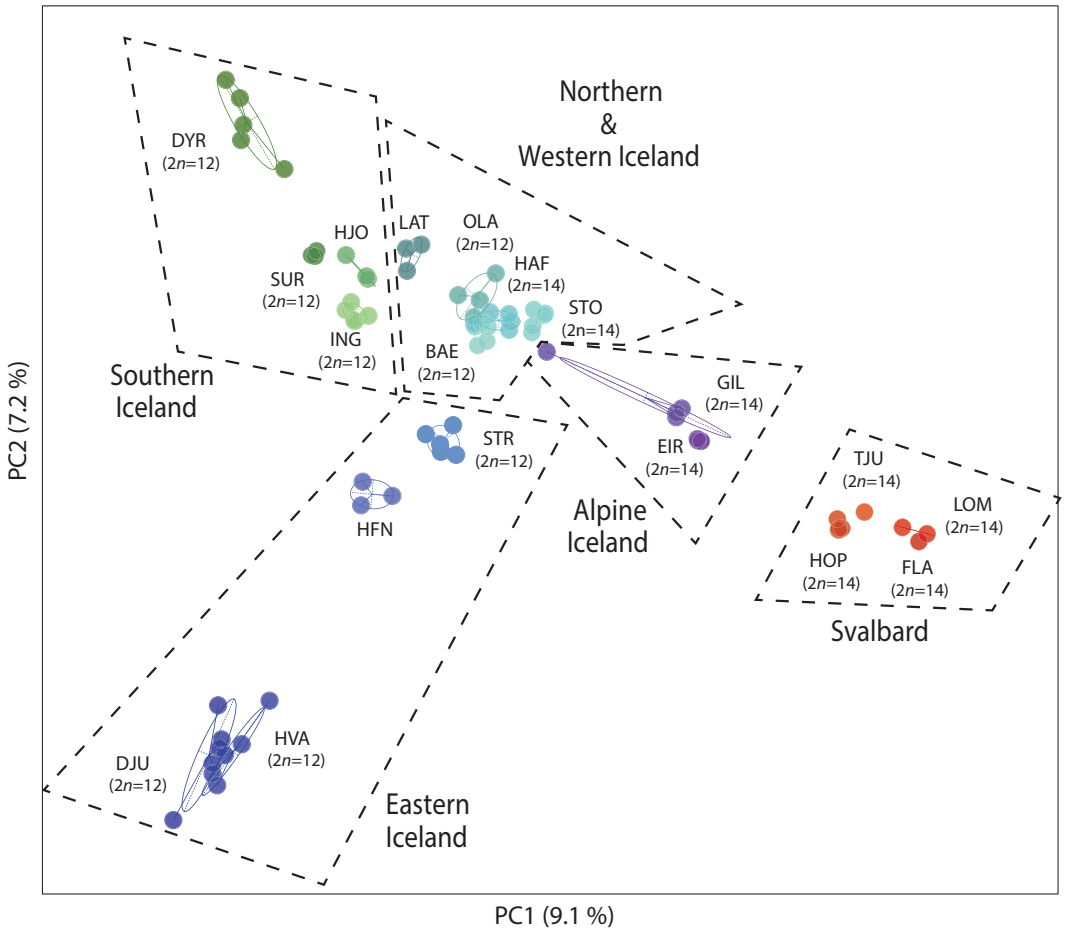

Figure 3. PCA, based on 1500 RADseq-derived SNPs, for 15 *Cochlearia* populations from Iceland and four populations from Svalbard. The first and second PC axes are visualized. Populations from southern Iceland are shown in green, populations from eastern Iceland in blue, populations from northern and western Iceland in turquoise, populations from alpine Iceland in purple and Svalbard populations in red. Chromosome numbers are indicated in brackets below the population abbreviation. For population abbreviations, see Table 1 and Supporting Information, Table S3.

the variance found among geographical groups and 15.14% among groups defined by chromosome number.

The lowest numbers of private alleles were found (1) between $2n = 14$ and $2n = 12$ populations from northern + western Iceland, (2) between alpine Icelandic ($2n = 14$) and Svalbard populations ($2n = 14$), and (3) between $2n = 14$ populations from northern + western Iceland and alpine Iceland (Fig. 4; Supporting Information, Table S4). No fixed private alleles (AA/aa or aa/AA) were found when comparing $2n = 12$ populations from southern, eastern and northern + western Iceland. Many private alleles were found when comparing Icelandic geographical groups with $2n = 12$ to Svalbard ($2n = 14$), but the highest numbers were found when comparing the Icelandic groups ($2n = 12$, 14) and Svalbard ($2n = 14$) to south-western Europe ($2n = 12$).

The heatmap based on allele frequencies (Supporting Information, Fig. S6) more or less confirmed the overall genetic structure based on SNPs, grouping together the alpine Icelandic populations with the Svalbard populations. The deviating sample from the alpine GIL population showed affiliation to the SUR population from southern Iceland, but gene flow between the two seems unlikely considering the barrier represented by the Vatnajökull and Eyjaflallajökull glaciers (see also the discussion about colonization of Surtsey from nearby areas). Among the coastal Icelandic populations, the southern Iceland populations (including SUR) grouped clearly together, whereas this was not as clear for other geographical groups. For instance, samples from HFN (north-eastern Iceland) and LAT (north-western Iceland) showed affiliation to different geographical groups.

Table 2. Analysis of molecular variance (AMOVA) based on 1500 RADseq-derived SNPs for 15 Icelandic *Cochlearia* populations: A, including populations of diploid *Cochlearia* from SW Europe (*C. aestuaria* and *C. pyrenaica*) and Svalbard (*C. groenlandica*), altogether 83 samples. B, Only Icelandic populations, altogether 65 samples. In both analyses, higher-level groups based on chromosome number ($2n$ = 12, 14) and geographical areas/groups are applied

Source of variation	d.f.	Sum of squares	Variance components	Percentage variance
A, All: 22 populations, 83 samples				
Among populations	21	4329.446	48.26407	65.76
Within populations	61	1532.650	25.12541	34.76
Chromosome number:				
2 groups: $2n$ = 12, $2n$ = 14				
Among groups	1	570.811	10.64995	13.14
Among populations within groups	17	3337.777	45.10731	55.66
Within populations	54	1365.233	25.28210	31.20
Geography:				
3 groups: SW Europe, Iceland, Svalbard				
Among groups	2	1117.081	26.84238	29.84
Among populations within groups	19	3212.365	38.00008	42.24
Within populations	61	1532.650	25.12541	27.93
B, Iceland: 15 populations, 65 samples				
Among populations	14	2659.855	38.93439	64.24
Within populations	50	1083.983	21.67367	35.76
Chromosome number:				
2 groups: 2n = 12, $2n$ = 14				
Among groups	1	432.238	10.17065	15.14
Among populations within groups	10	1844.568	35.68835	53.13
Within populations	43	916.267	21.30853	31.72
Geography:				
4 groups: Alpine, South, East, North and West				
Among groups	3	1143.363	15.35205	23.99
Among populations within groups	11	1516.492	26.97735	42.15
Within populations	50	1083.683	21.67367	33.86

Geography and environment

A weak isolation by distance was found to be significant (r = 0.283, P = 0.011) for the Icelandic samples (Supporting Information, Fig. S7A). Even though it was weak, a pattern could be observed where within-group comparisons as expected showed low genetic and low geographical distance. Otherwise, the comparison between alpine Iceland and eastern Iceland showed that some samples had high genetic distance despite low geographical distance, and the comparison between alpine Iceland and southern Iceland showed high genetic distance despite intermediary geographical distance. On the other hand, comparisons between eastern Iceland and northern + western Iceland showed intermediary genetic distance despite of large geographical distance.

Analysis of fine-scale spatial genetic structure showed that the data did not fit the null model (no spatial autocorrelation), i.e. the data displayed spatial autocorrelation (Fig. 5A). At short distances, the graph was higher than the expected null model and populations separated by these distances were expected to experience a considerable amount of gene flow. The distance where the graph crossed the null model, *c.* 130 km, was estimated to be the size of the genetic neighbourhood. At higher distances, the graph was less than what would be expected from the null hypothesis, with little or no gene flow occurring. Of the environmental (and elevation) variables, annual mean temperature and elevation had high absolute and significant correlation with ecotype/ecology (r = 1, P < 0.01 and r = –0.89, P < 0.01, respectively), whereas annual precipitation did not have significant correlation (r = 0.088, P = 0.75; Supporting Information, Fig. S7B). Isolation by environment was found to be significant when applying annual mean temperature (r = 0.422, P = 0.003; Fig. 5B).

MORPHOMETRY

The PCO analysis of leaf morphology clearly separated the alpine Icelandic populations (EIR and GIL) from the

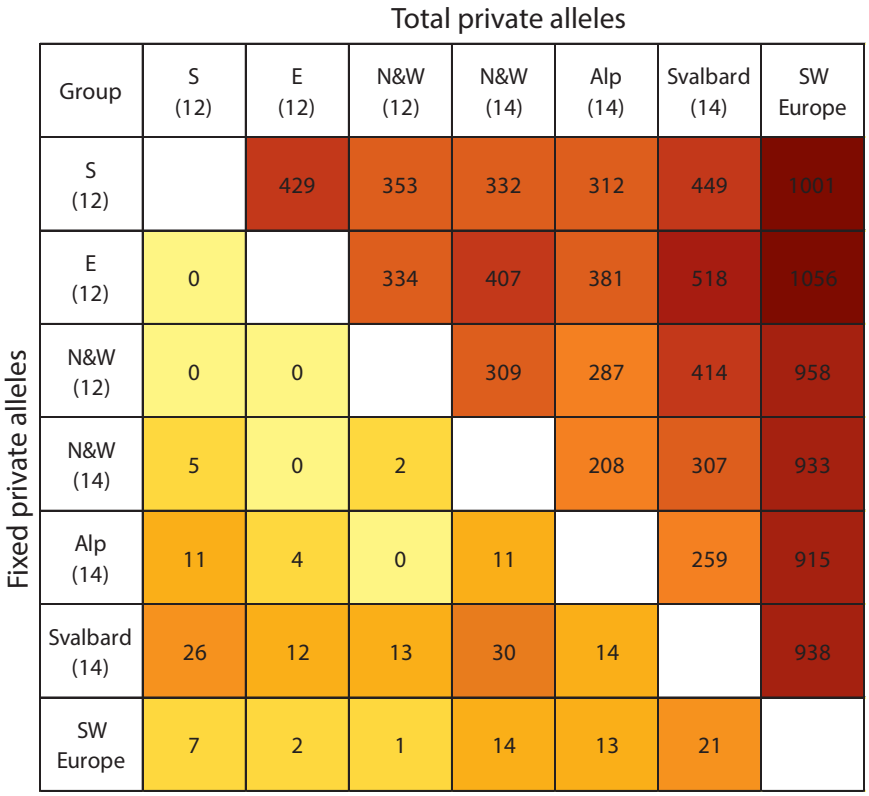

Figure 4. Total private alleles (upper triangle) and fixed private alleles (lower triangle) from pairwise comparisons of groups of *Cochlearia* samples (1500 RADseq-derived SNPs). Group name abbreviations: Alp = alpine Iceland, S = southern Iceland, E = eastern Iceland, N&W = northern and western Iceland. Chromosome number is given after group name. Fixed private alleles are homozygotes of the combination AA/aa or aa/AA.

coastal populations along PC1, which explained 78.6% of the variation in the cultivated dataset (Fig. 6A). Leaf morphology of the coastal population and of the geographical groups (southern, eastern and northern + western), overlapped considerably, but less so in the analysis based on field-collected material where southern and northern + western Iceland were separated along PC1 and eastern Iceland to some degree along PC2 (Supporting Information, Fig. S8).

There were significant differences among populations in all leaf traits for both field and cultivated material (Kruskal–Wallis test; Supporting Information, Table S5). Dunn's post hoc test (multiple comparisons) indicated that the alpine Icelandic populations were significantly different in all leaf traits compared to most of the coastal populations (Supporting Information, Table S5). This was seen as generally smaller and narrower leaves with cuneate leaf base (low values for leaf base angle) in the alpine plants compared to the larger and wider leaves with cordate leaf base in most of the coastal populations (Figs 6B, C; Supporting Information, Fig. S9, Table S6). The $2n = 14$ coastal populations (HAF, STO), which grow on volcanic rock, were closer to the alpine population in leaf size (and significantly different from several of the other coastal populations) based on field-collected data, but this was less pronounced after cultivation in controlled conditions (Fig. 6B, C; Supporting Information, Fig. S9, Tables S5, S6). Overall, differences in leaf length and leaf width were most pronounced in field-collected plants, whereas leaf ratio (leaf width/leaf length) and leaf base angle differentiated the alpine plants from the coastal plants just as well or even better after cultivation in controlled conditions (Supporting Information, Fig. S9).

Flower size based on field-collected material was significantly smaller in the alpine plants (EIR) compared to most of the coastal populations, but after cultivation in controlled conditions, no significant differences were seen for any of the flower traits (Supporting Information, Figs S10, S11, Tables S7, S8).

DISCUSSION

Plants with $2N = 12$ are common along the coast of Iceland

Most of the investigated *Cochlearia* plants in this study turned out to have a chromosome number of $2n = 12$.

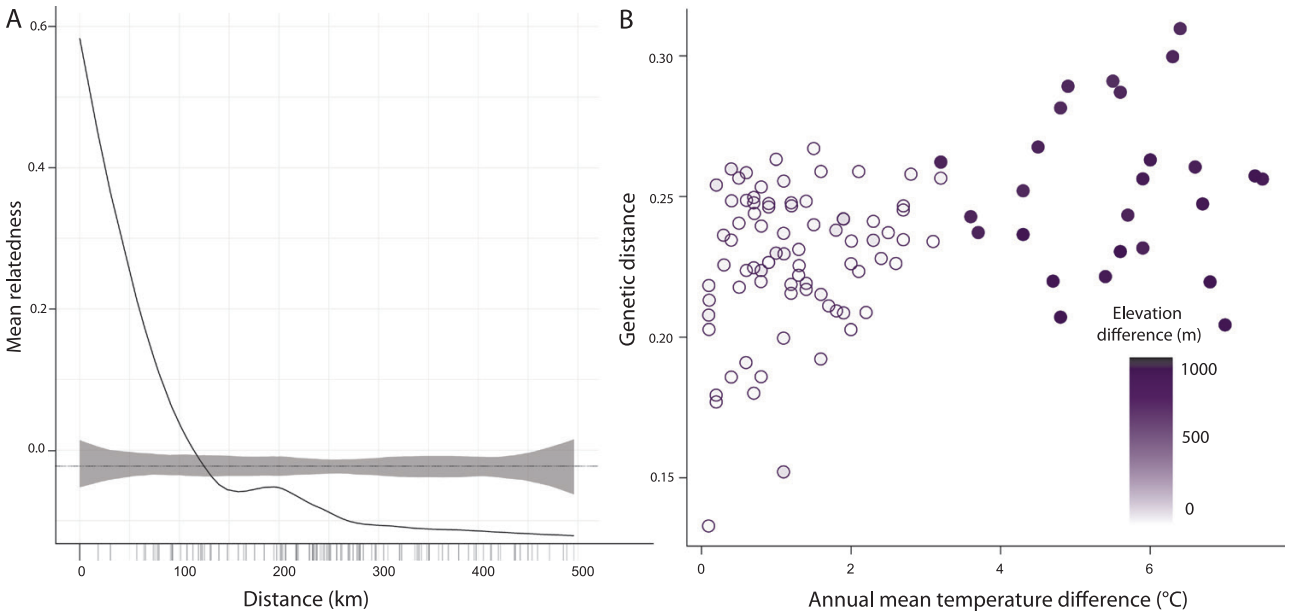

Figure 5. Visualization of fine-scale spatial genetic structure and isolation by environment for 15 populations of Icelandic *Cochlearia* based on 1500 RADseq-derived SNPs: A, Local polynomial fitting (LOESS) of pairwise relatedness to pairwise geographical distance. The null expectation is shown as the black dotted line with 95% confidence bounds with the shaded area. The LOESS fit to the observed relatedness is shown with the black solid line. The genetic neighbourhood size is estimated as the distance where the black solid line crosses the shaded area. B, Genetic distance (Euclidean) plotted against the annual mean temperature difference. Elevation difference is indicated by the shade of purple of the points, illustrating how the three factors relate.

This chromosome number, previously reported mainly from a few populations along the southern coast of Iceland (Gill, 1971; Nordal & Laane, 1990; Supporting Information, Table S1), seems to be more common in Iceland and the predominant cytotype along the coast. Only two of the sampled coastal populations (HAF and STO), both located in south-western Iceland, had chromosome number of $2n = 14$. Previously, coastal $2n = 14$ plants have in addition been reported from northern and north-western Iceland. The $2n = 14$ population Hafnir (HAF) in the south-west was sampled close to a site (Grindavíkurbjarg) where $2n = 12$ was previously reported (Löve, 1975), whereas the $2n = 12$ population from Ólafsvíkurenni (OLA) in western Iceland was sampled close to a site (Stapi) where $2n = 14$ was previously reported (Gill, 1971). This indicates that chromosome number may vary between geographically adjacent sites, and possibly even within a site. Stokkseyri in south-western Iceland is the only locality from which both chromosome numbers have been found so far ($2n = 14$: our study, Koch *et al.*, 1996; $2n = 12$: Wolf *et al.*, 2021). In the only population of this study, from which chromosome number from more than one individual was counted (BAE), all four individuals had $2n = 12$, but that does not necessarily warrant similar uniformity in all places. The two alpine populations had $2n = 14$, as also previously reported for the population at Eiríksstaðir (EIR; Nordal & Laane, 1990). No alpine population with $2n = 12$ has been observed in Iceland.

Based on analyses of the RADseq data, there is no clear genetic separation between the Icelandic *Cochlearia* plants purely based on cytotypes. The $2n = 14$ coastal populations from south-western Iceland (STO and HAF) are genetically more similar overall to coastal plants with $2n = 12$ than they are to alpine plants with $2n = 14$. Genetic admixture is found especially in the western and northern part of Iceland from where both chromosome numbers are reported. *Cochlearia* is known to hybridize even across ploidies with no or slightly reduced fertility in F1 and F2 generations (Crane & Gairdner, 1923; Saunte, 1955; Gill, 1971), and natural hybrids are found (Saunte, 1955; Gill, 1976). Likewise, high seed germination and fertility in the F1 generation were found when $2n = 12$ and $2n = 14$ plants were crossed in cultivation (Gill, 1971, 1973; Kenich, 2020).

Geographically structured genetic variation along the coast

The size of the genetic neighbourhood in this study is calculated from the genetic relatedness between pairs of individuals, used as a proxy for gene flow. The

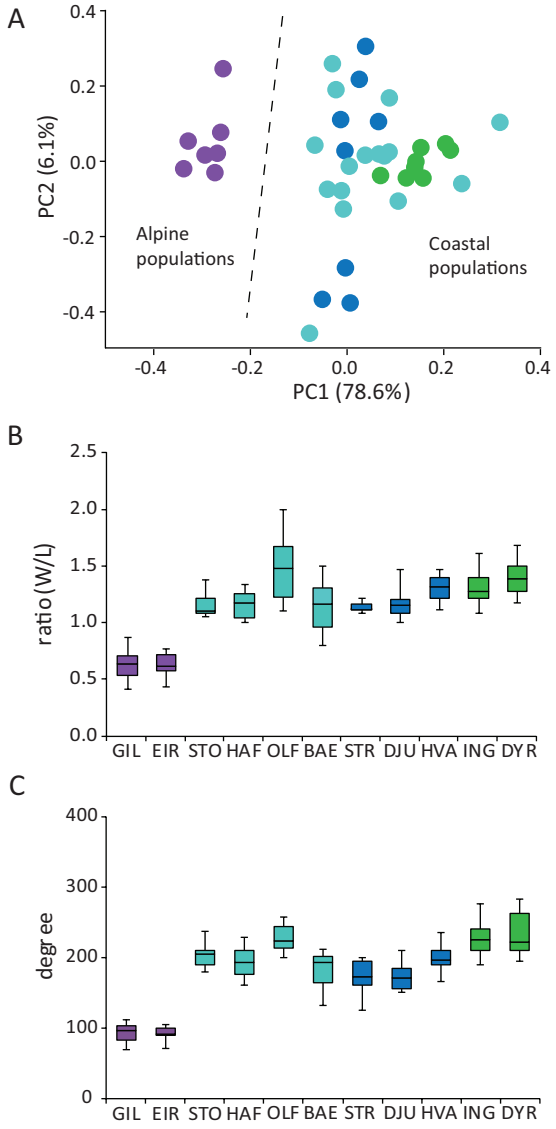

Figure 6. Variation in leaf morphology of 11 Icelandic *Cochlearia* populations after cultivation in controlled conditions. A, PCO analysis of leaf traits (leaf length, leaf width and leaf base angle). B, Boxplots of leaf ratio (leaf width/leaf length). C, Boxplots of leaf base angle. Colours represent geographical groups: southern Iceland (green), eastern Iceland (blue), northern and western Iceland (turquoise) and alpine Iceland (purple). Boxplots represent populations (for explanation of population abbreviations, see Table 1).

genetic relatedness represents the number of common ancestors in the recent past (Wang, 2017) and has been used widely to describe genetic connectivity (Monteiro *et al.*, 2019). Spatial autocorrelation was detected among the *Cochlearia* populations, supported by a genetic neighbourhood size of *c.* 130 km. Populations separated below this distance experience higher gene flow, whereas populations separated above this distance experience lower gene flow. This fits well with the rough geographical groups around the Icelandic coast indicated by the STRUCTURE results. In further detail, populations from the south-west (HAF and STO) through the northern part of the island and all the way to the northernmost eastern populations appear more connected despite relatively long geographical distances, whereas the southern group and the two southernmost eastern populations (DJU and HAV) form two quite distinct groups with little or no admixture. The functional connectivity in plants is defined as 'the effective dispersal of propagules/pollen among habitat patches in a landscape' (Auffret *et al.*, 2017). This leaves two possible explanations for the observed pattern: directional dispersal or stretches of unsuitable habitat along the coast of Iceland.

Exposed coastal cliffs, where *Cochlearia* often grows, are a widely distributed habitat along the Icelandic shoreline. In the south, there are, however, several long glacier outwash plains, i.e. sandurs, that are sheltered deltas with fine-grained material. These stretches seem to be less populated by *Cochlearia*, whereas the rest of the coast is more or less continuously populated (GBIF Secretariat, 2021). Apart from unsuitable habitat resulting in patchier populations with reduced gene flow in the south, directional dispersal by coastal sea currents and/or seabirds might also be relevant to explain the observed genetic patterns. The position of Iceland in the ocean results in different Atlantic and Arctic sea currents meeting and directing the water flow around the island. Logemann *et al.* (2013) proposed a three-dimensional circulation scheme where the South Icelandic Current flows westwards along the southern coast and then breaks off from Iceland, continuing further south. From the influence of the Irminger Current, a current starting in the south-west flows northwards along the western coast of Iceland where, when it meets currents from the north, it continues eastwards along the northern coast and southwards along the eastern coast, before breaking off to the east. The genetic admixture observed in western and northern Iceland and the genetic boundary between populations in southern and south-western Iceland might, thus, both be related to the main ocean currents around Iceland.

Whereas dispersal influenced by sea currents has been found for other coastal plants, e.g. *Cakile maritima* Scop. (Brassicaceae) (Gandour, Hessini & Abdelly, 2008), floating experiments of *Cochlearia* seeds indicate that dispersal with sea currents is probably limited to shorter distances (Praeger, 1913; Quinn *et al.*, 1994). Brandrud *et al.* (2017) argued that limited ocean dispersal corresponds well with strong population affiliation and limited gene exchange of the *C. officinalis* populations in northern Norway.

However, the situation could be different in Iceland, and dispersal by sea currents might proceed in short steps and directionally follow the relevant current. *Cochlearia* was among the earliest plants to colonize the volcanic island Surtsey after it originated off the southern coast of Iceland in 1963 (Fridriksson, 1987; Baldursson & Ingadóttir, 2007). Initially *Cochlearia* probably colonized Surtsey by marine dispersal (first registered in 1969; Fredriksson, 2000; Magnusson, Magnusson & Fridriksson, 2009), but it was not until the establishment of the first seagull colony in 1985 that it really took a foothold, taking advantage of nitrogen rich conditions around the colony (Baldursson & Ingadóttir, 2007). Seagulls were probably also dispersal agents as pellets regurgitated by the birds at the breeding site turned out to consist mostly of *Cochlearia* material (Fridriksson, 2000). In the colonization sequence and location of new species, *Cochlearia* is considered the first to be carried by birds to the island (Magnusson *et al.*, 2009). Most of the species on Surtsey are believed to come from immediate neighbouring islands or the Iceland mainland < 35 km away (Fridriksson, 1987; Baldursson & Ingadóttir, 2007), although occasional longer distance dispersal by birds has also been suggested (Fridriksson, 1987). The spatial genetic structure of *Honckenya peploides* (L.) Ehrh. thus indicates multiple colonization events to Surtsey from the nearby island Heimaey and from the southern coast of Iceland (Árnason *et al.*, 2014). Whereas sea birds are ultimately in control of their own movement, it has been shown that their movement patterns are also influenced by wind and sea currents (Shamoun-Baranes *et al.*, 2011; Yoda, Shiomi & Sato, 2014; Cooper *et al.*, 2018). Razorbills (*Alca torda*) tend to rest on the sea overnight, shearwaters spend half of their time as passive drifters and seagulls also rest drifting on the sea.

Environmentally structured genetic variation

Significant isolation by environment (applied as annual mean temperature) with elevation may be used to describe the genetic difference between coastal and alpine habitats. The isolation of the alpine populations is supported by the genetic boundary analysis; however, the isolation is not complete as the two alpine populations seem to be genetically linked towards the coastal populations in northern + western Iceland, including the two $2n = 14$ populations. This is supported also by a lower number of private alleles when comparing the two alpine populations to the northern + western populations, than when comparing them to the remaining coastal populations.

One explanation for this connection could be common descent of the $2n = 14$ plants, followed by later isolation leading to genetic divergence. The genetic homogeneity and low admixture of the alpine populations suggest that ongoing gene flow with the coastal populations is unlikely, probably owing to the topography, with elevation functioning as an effective barrier and limiting contact between plants in the two habitats to infrequent long-distance dispersal events. The aberrant and admixed sample from the Gilsbakki (GIL) population could be a possible result of long-distance dispersal (even though we cannot fully rule out the possibility of laboratory contamination considering that we only found signs of admixture in a single sample). Despite the close genetic affinity between the two alpine populations, it seems unlikely that they have ongoing gene flow as the MIB constitutes an efficient barrier, which in other plant groups, such as *Betula* L. (Thórsson *et al.* 2010), has contributed to east–west phylogeographical patterns.

Iceland, with most of its land mass situated just south of the Arctic Circle, is the only place where the $2n = 12$ and $2n = 14$ *Cochlearia* cytotypes co-occur (Nordal & Laane, 1990). Coastal habitats in Iceland offer a temperature range close to the temperature range of the mountainous springs that *C. pyrenaica* ($2n = 12$) inhabits in mainland Europe (Fig. 1). The alpine habitat in Iceland is closer to the temperature range of the Arctic, where *C. groenlandica* ($2n = 14$) occurs. The harsh conditions in alpine habitats may be a challenge to the plants, particularly since *Cochlearia* has no dormancy phase in winter. Accordingly, vegetative rosettes must endure extreme temperatures, wind, long-lasting snow cover (Gill, 2007) and a short growing season (Nordal & Laane, 1990). Birkeland *et al.* (2020) found candidate genes for adaptation to cold (stress) in the Arctic *C. groenlandica* ($2n = 14$) compared to *C. pyrenaica* ($2n = 12$) from mainland Europe. This supports that environment (temperature in this case) seems to be an important factor to explain the distribution of *Cochlearia* in Iceland.

Ecological differentiation from coast to inland is also observed at the tetraploid level in *Cochlearia* in northern Norway (Nordal & Laane, 1996; Brandrud *et al.*, 2017). In that system, the observed differentiation between ecotypes is probably a result of local adaptation and recurrent parallel ecotypic diversification from the ancestral beach ecotype, potentially representing an early stage in the process of speciation (Brandrud *et al.*, 2017). Subject to the fact that only two alpine populations were included in our analyses, the environmentally structured genetic variation seen in Iceland seems not to represent a parallel example of recurrent and polytopic ecotype divergence.

In support of the results from the genetic data, there are also morphological differences between coastal and alpine populations. The observed morphological traits correspond to what Nordal & Laane (1990)

reported: coastal plants have larger cordate/reniform leaves whereas alpine plants have small lanceolate leaves with a cuneate base. These morphological differences were retained after cultivation in controlled conditions, thus indicating underlying genetic factors as suggested by Nordal & Laane (1990). The overall smaller alpine plants might, thus, be a result of genetic adaptation to the more severe habitat and shorter growing season to which they are exposed (Billings & Mooney, 1968). However, the differences in size were less pronounced in controlled conditions, and the overall boosted growth in growth chambers indicates that environmental factors limit the size of wild plants (Nordal & Stabbetorp, 1990; Pegtel, 1999; Gill, 2007).

Geographical or environmental patterns observed in leaf size and form among field-collected coastal populations appear to be plastic responses, since these patterns were not upheld in controlled conditions. In field conditions, leaf traits of the coastal $2n = 14$ populations (HAF and STO) were closer to the alpine populations (with overall smaller leaf size), but not significantly so after cultivation. The smaller leaves could be an environmental effect of the volcanic rock habitat where these two populations grow. In field-collected plants, petal length and width separate the coastal and alpine populations, but these differences were not observed after cultivation in controlled conditions and is probably also a plastic trait.

Although leaf morphology, especially petiole length and leaf size, has been noted to be highly variable and uncertain comparative traits in *Cochlearia* (Pegtel, 1999), leaf base is considered to be less biased by environmental factors and a more reliable trait that can be used taxonomically to distinguish species (Nordal & Laane, 1990, 1996; Nordal & Stabbetorp, 1990; Pegtel, 1999). Nordal & Laane (1990) also used petal length and seed size in combination to morphologically differentiate between the coastal and alpine plants in Iceland. The present study finds leaf base to be a reliable trait, whereas floral traits appear biased by environmental factors. Seed size was not measured.

Two *Cochlearia* species in Iceland?

The name *C. officinalis* has been used for Icelandic *Cochlearia*, with or without subspecies, by several authors (Löve, 1945; Stefánsson, 1948; Kristinsson, 2010, 2018). Plants of *C. officinalis* from the Britain, Ireland and Scandinavia were not included in the present study, but data from Wolf *et al.* (2021) suggest these are genetically divergent from the Icelandic plants. This together with difference in ploidy (tetraploid vs. diploid) and mating system suggests that *C. officinalis* is not an appropriate name for the Icelandic plants. A pilot project showed that *Cochlearia* plants from Iceland and Svalbard were self-compatible with 100% seed set after assisted self-pollination (Nordal & Laane, 1990). Auto-deposition resulted in 100% seed set in the Svalbard and alpine Icelandic populations ($2n = 14$), but in somewhat reduced seed set (17–100%) in the coastal Icelandic populations ($2n = 12, 14$). This is in contrast to populations of *C. officinalis* along the Scandinavian coasts ($2n = 24$), which is completely self-incompatible (Nordal & Stabbetorp, 1990). The Scandinavian plants, thus depending on insect pollination, differ from the Svalbard/Icelandic plants by having larger flowers, producing large amounts of nectar and accordingly smelling strongly of honey.

Coastal $2n = 12$ Icelandic plants have previously also been referred to as *C. pyrenaica* (Löve, 1975). *Cochlearia pyrenaica* is mainly distributed in alpine regions of mainland Europe from the Pyrenees to Ukraine, but it has a few suggested northern occurrences in Britain (Nordal, 1988). A close relationship between the Icelandic $2n = 12$ plants and *C. pyrenaica* in mainland European is, however, not supported genetically in this study. Wolf *et al.* (2021) included material from England and Scotland in their genomic analyses, indicating that there is a considerably larger split between the British and the Icelandic plants than there is between the British plants and *C. pyrenaica* from mainland Europe. This might, thus, present a good case for resurrecting the name *C. islandica*, which has already been suggested for $2n = 12$ populations in Iceland (Pobedimova, 1970), in line with the latest annotation by the Icelandic Institute of Natural History (Wasowicz, 2020). More thorough genetic and morphological analyses are, however, needed before firm conclusions can be made.

The two alpine $2n = 14$ populations investigated here are, on the other hand, genetically similar to the Svalbard populations, despite being separated by 1950 km of the Arctic Ocean, and could well be included in *C. groenlandica*, as also suggested by Wasowicz (2020). The observed genetic pattern might be due to lineage separation. Individuals belonging to the same species are commonly closely related despite large geographical separation, whereas individuals belonging to different species would be different even when they are closely located (Duminil & Michele, 2009; Medrano, Herrera & Bazaga, 2014). We did not include plants of *C. groenlandica* from eastern Greenland, but in another study (Bruholt, 2019) RADseq data from eastern Greenland and Svaldbard were grouped closely together, and these were clearly separated from *C. groenlandica* from western Greenland. Separation between the western and eastern parts of the North Atlantic region and a close connectivity in the eastern part is a common pattern found in several other studies of the genetic structure in Arctic plant species, e.g. *Cassiope tetragona* (L.) D.Don (Eidesen *et al.*, 2007) and *Salix herbacea* L. (Alsos *et al.*, 2009).

Cochlearia groenlandica in Svalbard is reported with two growth forms, with tall vigorous plants in the vicinity of nutrient-rich bird cliff colonies and smaller plants on more nutrient-poor tundra substrate (Nordal & Laane, 1990; Zmudczyńska-Skarbek *et al.*, 2013). Similar luxuriant growth in bird cliff plants of *C. officinalis* has been shown to result from an efficient uptake of nitrogen and possible adaptation to nutrient-rich habitats (Eriksen & Nordal, 1989). Plants from Svalbard and Greenland were not included in the morphological analysis in this study, but other studies have shown that the tundra plants in Svalbard and eastern Greenland are morphologically similar to alpine Icelandic plants (Nordal & Laane, 1990; Bruholt, 2019). The late snowbed habitat, where we collected the Icelandic alpine plants, has much in common with the Arctic tundra where *C. groenlandica* grows in Svalbard. Because of the high latitude, the environmental conditions on Svalbard are severe, even during the summer, and plants are exposed to stress, such as low nutrient and water availability, short growing season, unstable weather conditions and permafrost (Zmudczyńska-Skarbek *et al.*, 2013). These habitats are also characterized by low availability of pollinators and self-reproduction, as found in alpine Icelandic and Svalbard *Cochlearia* plants (Nordal & Laane, 1990), may be a huge advantage.

Origin of the two cytotypes

Based on cytogenetic studies, Gill (1971, 1973) argued that the $2n = 14$ *C. groenlandica* could have originated by primary tetrasomy from the $2n = 12$ group, as he found an extremely high frequency of trivalent formation in hybrids between the two cytotypes, no cells with three univalents and no closed-ring trivalents. Indeed, production of disomic gametes ($n + 1$) in $2n = 12$ plants and their subsequent union may result in tetrasomic progeny ($2n = 12 + 2$). Although the tetrasomic individual would suffer from reduced fertility and imbalanced gene content (Otto, 2007), recombination with non-homologous chromosomes may eventually stabilize the complement and restore fertility. Iceland has previously been suggested as the place of origin for a transition from $2n = 12$ to $2n = 14$ (Nordal & Laane, 1990). Such a scenario would indicate that the $2n = 14$ cytotype has dispersed from Iceland into the Arctic to obtain its currently wide distribution. Studies on floristic composition, genetic data and ancient sedimentary DNA suggest that the Britain and Ireland were an important source area for vascular plants during the Pleistocene colonization of Iceland (Eidesen *et al.*, 2013; Alsos *et al.*, 2015, 2021). The genetic connections of *Cochlearia* in Britain and Ireland towards both Iceland and south-western and central European diploid ($2n = 12$) *Cochlearia* fit the commonly observed pattern for several plant species with survival south of glaciated areas followed by colonization northward (Schönswetter *et al.*, 2003; Schönswetter, Popp & Brochmann, 2006). The seemingly higher diversity of the $2n = 12$ gene pool indicated by our RADseq data could thus be explained by reduced genetic diversity in the northern populations as a consequence of a trailing-edge scenario (Hewitt, 1996). The observed patterns could, of course, also result from other past or ongoing demographic processes, and more thorough phylogeographic analyses are needed.

Further, Mandáková *et al.* (2017a) analysed *C. pyrenaica*, one of the species with the lowest chromosome number in the genus ($n = 6$), and provided convincing cytogenetic and transcriptomic evidence that the most recent common ancestor of *Cochlearia* had a hexaploid genome. The ancestral hexaploid chromosome number can be inferred to be between $2n = 36$ and $2n = 48$ (purporting alternative base numbers $n = 6$, 7 and 8). Although a detailed whole-genome analysis of a diploid species of *Cochlearia* is lacking, Mandáková *et al.* (2017a) proposed that the extant chromosome number variation among the diploid genomes resulted from post-polyploid structural diploidization including the reduction of chromosome number (descending dysploidy). Hence, the extant chromosome complements of $2n = 12$ and $2n = 14$ may represent results of independent descending dysploidies from the ancestral hexaploid number, and thus, 12-chromosome plants may be evolutionary younger derivatives of 14-chromosome plants if the one-way directionality of descending dysploidy is assumed. Post-polyploid dysploidies are reported in numerous (meso)polyploid Brassicaceae clades and species (e.g. Mandáková *et al.*, 2013, 2017a, 2017b), reviewed by Mandáková & Lysak (2018). In line with a step-by-step descending dysploidy and based on a time-calibrated phylogenetic tree produced from plastid DNA sequences, Wolf *et al.* (2021) suggest that $2n = 14$ gave rise to $2n = 12$, as they found that the earliest diverging organellar genomes of known diploids were *C. groenlandica* ($2n = 14$) from western North America (British Columbia and Alaska). These authors argued that from a phylogenetic point of view, the most parsimonious scenario recognizes a base chromosome number of $n = 7$ as the ancestral state. However, future comparative whole-genome sequencing of both Icelandic cytotypes should be performed to uncover the phylogenomic relationships between two chromosome variants.

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DATA AVAILABILITY

Data underlying results in this article are available as (1) a file with SNPs derived from RADseq data and (2) a file with morphological raw data. Both files can be accessed in https://doi.org/10.5061/dryad.d51c5b04s.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Previously published chromosome numbers (2*n*) for *Cochlearia* in Iceland
Table S2. Overview of the taxonomic treatment and nomenclature used for Icelandic *Cochlearia* plants by different authors
Table S3. Locality, collection and voucher information for reference populations of diploid *Cochlearia* from Svalbard and south-western Europe
Table S4. Private alleles in pairwise comparisons between geographical groups of *Cochlearia*, based on 1500 RADseq-derived SNPs
Table S5. Test output for differences in leaf traits among field-collected and cultivated plants from Icelandic *Cochlearia* populations
Table S6. Summary statistics of leaf traits in field-collected and cultivated plants from Icelandic *Cochlearia* populations
Table S7. Test output for differences in flower traits among field-collected and cultivated plants from Icelandic *Cochlearia* populations
Table S8. Summary statistics of flower traits in field-collected and cultivated plants from Icelandic *Cochlearia* populations
Figure S1. Illustration depicting approximately how measurements were made in leaf morphometric analyses of *Cochlearia* plants.
Figure S2. Mitotic chromosomes of nine Icelandic *Cochlearia* populations.
Figure S3. Mitotic chromosomes of three additional *Cochlearia* plants from the population Bær á Höfðaströnd (BAE) in northern Iceland.
Figure S4. Results from STRUCTURE analysis based on 1500 RADseq-derived SNPs, for populations of Icelandic *Cochlearia* and diploid *Cochlearia* from Svalbard and south-western Europe.
Figure S5. PCA based on 1500 RADseq-derived SNPs, for 15 *Cochlearia* populations from Iceland and four populations from Svalbard.

Figure S6. Heatmap and network generated from allele frequencies (1500 RADseq-derived SNPs) for Icelandic *Cochlearia* populations and diploid *Cochlearia* from Svalbard and south-western Europe.
Figure S7. Mantel test of isolation by distance between geographical groups of Icelandic *Cochlearia*, and correlation of environmental variables to the ecotype variable (coast or alpine).
Figure S8. PCO analysis of leaf traits in 11 field-collected Icelandic *Cochlearia* populations.
Figure S9. Boxplots of leaf traits in Icelandic *Cochlearia* populations, from field-collected and cultivated material.
Figure S10. Boxplots of flower traits in Icelandic *Cochlearia* populations, from field-collected and cultivated material.
Figure S11. PCO analyses of flower traits in field-collected and cultivated plants from Icelandic *Cochlearia* populations.